PHARMACOLOGIST'S REVIEW

BLA: 97-1325
SPONSOR: Seragen, Inc.
PRODUCT: recombinant interleukin-2 diphtheria toxin fusion protein; $DAB_{389}IL-2$; denileukin diftitox injection; ONTAK™
FORMULATION/CHEMISTRY: an *E. coli* derived recombinant IL-2 receptor (IL-2R) specific cytotoxin, which is a purified protein with a MW of 58 kD. The fusion protein is composed of the amino acid sequences ——— for diphtheria toxin fragments A [enzymatically active] and B [membrane translocationl, followed by the sequences for IL-2 [——————————————————— ——————— Neomycin, used in the fermentation process, is undetectable in the final product. Supplied in single use vials as a sterile, clear liquid intended for IV injection. Each vial contains 2 ml of 150 µg/mL $DAB_{389}IL-2$ in 20 mM citric acid, 0.05 mM EDTA, <1% polysorbate 20 in Water for Injection, USP, pH 7.1
PROPOSED INDICATION: $DAB_{389}IL-2$ is designed to direct the cytocidal action of DT, in a site-specific manner, to malignant cells that express the IL-2R. Such cells are found in certain leukemias and lymphomas, such as cutaneous T-cell lymphoma. ONTAK™ is indicated for the treatment of patients with CTCL which is persistent or recurrent despite prior therapy.
ABBREVIATIONS: recombinant interleukin-2 diphtheria toxin fusion protein = $DAB_{389}IL-2$; diphtheria toxin = DT; cutaneous T-cell lymphoma = CTCL; subcutaneous = SC; intravenous = IV; platelet = PLT; bone marrow = BM

assigned 12/18/97; received 12/27/97; completed; 5/14/98

CROSS-REFERENCES: IND ———————

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INTRODUCTION:

CTCL is a general term for a constellation of low grade non-Hodgkin's lymphomas in which malignant T-cells are initially present in the skin. Mycosis fungoides and its erythrodermic, leukemic variant, Sézary syndrome, are the most common forms of CTCL and patients with both of these forms were included in clinical trials. CTCL initially involves only the skin, but eventually progresses to include LNs, spleen, liver, and/or other viscera. The disease is disfiguring and debilitating, thus the quality of life for the patient is compromised. There is often intense and unremitting pruritus. In addition, due to disruption of the skin barrier, recurrent episodes of skin infections occur in these patients. Once the disease has progressed beyond Stage Ia, spontaneous remissions generally do not occur. There are no current therapies in place that can cure CTCL. The median survival for CTCL patients is 8-10 years, with those patients having visceral or LN involvement having a median survival time of <3 years.

This fusion protein utilizes the cytotoxic action of DT, as well as the cell targeting ability for the IL-2R. Following the binding of the mature fusion protein of 58 kD to the IL-2R on cells, the protein is [redacted] . and processed by a [redacted] . This results in [redacted] and resulting in cell death. The first fusion protein developed by Seragen was $DAB_{486}IL\text{-}2$. This protein was used in Phase I/II clinical trials (124 patients, including 36 with CTCL). A more potent fusion protein - $DAB_{389}IL\text{-}2$ - was formulated, and following preclinical testing of both agents, the present product was used in clinical trials.

$DAB_{389}IL\text{-}2$ has been granted orphan drug designation for the treatment of CTCL by FDA Office of Orphan Products Development. CBER agreed to an accelerated approval designation for this treatment based upon the significant responses in CTCL patients who have received prior therapy. CBER also agreed to the qualification of the ongoing placebo-controlled study in patients with fewer prior therapies as a Phase IV commitment.

The proposed clinical indication [per the package insert] for $DAB_{389}IL\text{-}2$ is for the treatment of patients with CTCL which is persistent or recurrent despite prior therapy. The proposed treatment regimen [per the package insert] is IV infusion (at least 15 mins) of [redacted] µg/kg/day ONTAK™ for 5 consecutive days. Courses may be repeated every 21 days [the maximum number of courses given clinically was 11].

Formulation - Two formulations have been used in clinical trials. Initially, ——— was used, but the formulation did not allow for long-term storage at ≥-20°C. A citrate-EDTA formulation is the formulation for licensure [a biocomparability study was performed in humans with both formulations].

In July 1994, the measurement of protein was changed from the ——— assay to the ——— (chromatographic) assay. For all preclinical studies, the sponsor has recalculated the values based on ——— (size exclusion chromatography) measurement.

Preclinical Pharmacology Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

In vitro

1. In Vitro Studies to Determine the Mechanism of Action and Specificity of DAB_{389}IL-2 with Comparisons to DAB_{486}IL-2: A Summary Report; report #94032; performed at Seragen (non GLP); lot ——— (DAB_{389}); ——— (DAB_{486}); 7/94; vol 10

2. PA1 No. IM091 - Immunohistochemical Localization of Rat IL-2 Receptor in Selected Tissues Using ———; report #94130; performed at ——— (non-GLP); lot #unknown; 8/94; vol 12

3. PA1 No. IM120 - Immunohistochemical Localization of IL-2 Receptor in Selected Human Tissues; report #94131; performed at ——— (non-GLP); lot #unknown; 8/94; vol 12

4. Generation of an Active Metabolite of DAB_{389}IL-2 by Proteolytic Cleavage Between the Active and Membrane Associating Domains: A Summary Report; report #95042; performed at Seragen (non-GLP); lot ——— 5/96; vol 13

5. In Vitro Studies Comparing the Mechanism of Action of Native DAB_{389}IL-2 and Trypsin-Nicked DAB_{389}IL-2 on Tumor Cells and on Activated Normal Lymphocytes; report #95084; performed at Seragen (non-GLP); batch ——— 2/96; vol 13

6. In Vitro Studies on the Interaction of DAB_{389}IL-2 with Human CD4+ Lymphocytes and CD8+ Lymphocytes; report #95148; performed at Seragen (non-GLP); lot ——— 4/96; vol 14

7. Investigation into the Potential for Interaction of [^{35}S]DAB_{389}IL-2 with a High Molecular Weight Entity in Serum; report #96009; performed at Seragen (non-GLP); lot ——— ——— [radioactive form]; ——— [nonradioactive f orml; 6/96; vol 15

8. In Vitro Studies on the Mechanism of DAB_{389}IL-2 Induced Cell Death; report #96011; performed at Seragen (non-GLP); lot ——— [DAB_{389}IL-2]; ——— 4/97; vol 15

9. Stability of DAB,,IL-2 in Human Serum and Rat Serum-and Plasma; report #96014; performed at Seragen (non-GLP); lot ——— 6/96; vol 15

10. Evaluation of DAB_{389}IL-2 Cytotoxicity and IL-2R Expression in a Panel of Primary Human Cell Types; report #96066; performed at Seragen (non-GLP); lot ——— 1/97; vol 20

11. Characterization of the Specificity, Kinetics, and Mechanism of DAB_{389}IL-2 Cytotoxicity and Correlation with IL-2 Receptor Expression in a Panel of Tumor Cell Lines; report #96067; performed at Seragen (non-GLP); lot ——— 1/97; vol 20

12. Effect of DAB_{389}IL-2 After 20-Hour Exposure in Human Liver and Kidney Slices; report #96068; performed at ——— (per GLP); lot #unknown; 4/96; vol 20

13. In Vitro Inhibition of Protein Synthesis by DAB_{389}IL-2 in Primary Cultures of Rat Hepatocytes, Kupffer Cells, and Renal Proximal Tubule Epithelial Cells; report #96111; performed at Seragen (non-GLP); lot ——— 1/97; vol 20

14. Evaluation of the Impact of DAB,,,IL-2 on Primary Cultures of Human Endothelial Cells; report #96114; performed at Seragen (non-GLP); lot ——— (DAB_{389}IL-2), ——— 1/97; vol 20

15. IL-2R Contact Time Studies with Activated Human CD8+ and CD4+ Lymphocytes; report #96154; performed at Seragen (non-GLP); lot ——— 3/97; vol 22

16. Assay Development and Performance Report: ELISAs for the Determination of Anti-DAB_{389}IL-2, Anti-Diphtheria Toxin, and Anti-IL-2 Ab Titers in Human, Monkey, Rat, and Mouse Serum; report #94177; performed at Seragen (non-GLP); lot #unknown; 5/97; vol 22

17. Assay Development and Performance Report: Determination of DAB_{389}IL-2 Levels by ELISA with Anti-fragment B Capture and Chemiluminescent Detection; report #95021; performed at Seragen (non-GLP); lot #unknown; 6/96; vol 22

18. Assay Development and Performance Report: Determination of DAB_{389}IL-2 Bioactivity Levels by-Bioassay; report #95032; performed at-Seragen (non-GLP); lot ——— 8/96; vol 22

19. Assay Validation Report: Determination of DAB_{389}IL-2 Bioactivity Levels in Preclinical and Clinical Serum Samples by Bioassay; report #95054; performed at Seragen (non-GLP); lot #unknown; 6/96; vol 22

20. Assay Development and Performance Report: Determination of Neutralizing Anti-DAB_{389}IL-2 Antibody Levels: report #96098; performed at Seragen (non-GLP); lot ———. 5/97; vol 22

21. Measurement of DAB_{389}IL-2 Blood Levels; report #96113; performed at Seragen (non-GLP); lot #unknown; 10/96; vol 22

In vivo

1. Impact of Treatment with DAB_{389}IL-2 on Preimmune and Naive C57BL/6 Mice in the CP3 Model of IL-2R Expressing Malignancy; report #95126; performed at Seragen (non-GLP); lot ——— 4/97; vol 14

2. Impact of Dose and Schedule of DAB_{389}IL-2 in a Murine Model of IL-2 Receptor Expressing Malignancy: A Summary Report; report #96012; performed at Seragen (non-GLP); lot ——— 6/96; vol 15

3. Specificity of DAB_{389}IL-2, ——— and ——— in Murine Models of IL-2R Expressing and IL-2R Negative Malignancy; report #96055; performed at Seragen (non-GLP); lot ——— ——— : 8/96; vol 16

4. Adoptive Transfer of Lymph Node Cells from CP3-Injected Mice: Evaluation of Subsequent Tumor Development and Sensitivity to DAB_{389}IL-2; report #96069; performed at Seragen (non-GLP); lot #unknown; 5/97; vol 20

Reports Not Included in the BLA (from IND ———

1. Impact of DAB,,IL-2 in a Murine Model of IL-2R-Expressing Malignancy: A Summary Report; report #94031; performed at Seragen (non-GLP); lot #unknown

Pharmacology Studies

In vitro

1. In Vitro Studies to Determine the Mechanism of Action and Specificity of DAB,,, IL-2 with Comparisons to DAB_{486}IL-2: A Summary Report

Findings: DAB_{389}IL-2 is a potent cytotoxic agent for cell lines and activated lymphocytes which express the high affinity IL-2R (lowest IC,, = 2 x 10^{-12} M). Cells that do not-express IL-2R or express the p55 or only the p75 and p64 subunits of the IL-2R, are not sensitive to the fusion protein. In addition, DAB_{389}IL-2 was equally cytotoxic for T and B cells of human, monkey, rat, and mouse origin that expressed IL-2R. DAB_{389}IL-2 was 4 to 30-fold more potent than DAB,,, IL-2, depending on the cell type used. The sponsor attributes this higher potency to the greater affinity of DAB,,, IL-2 for the high affinity IL-2R & the shorter contact time needed for irreversible binding to the receptor. The kinetics of the inhibition of protein synthesis were similar for both materials.

2. PA1 No. IM091 - Immunohistochemical Localization of Rat IL-2 Receptor in Selected Tissues Using [redacted]

Tissues evaluated - adrenal, kidney, liver, lung, LN, spleen

Findings:
Specific staining of lymphocytes was intense, suggestive of localization of the receptor to the cell membrane & cell processes. The stained lymphocytes were located in & near lymphoid tissues (BALT, LN, spleen) and at sites of inflammation (kidney, periportal regions of liver).

3. PA1 No. IM120 - Immunohistochemical Localization of IL-2 Receptor in Selected Human Tissues
[used a mouse IgG1 against human IL-2R]
Tissues evaluated - adrenal, kidney, liver, lung, LN, spleen

Findings:
Moderately, but specifically stained lymphocytes (i.e., indicative of IL-2R presence) were located in the cell membrane of lymphoid tissues (LN, spleen) and at sites of lymphoid aggregates (periportal regions of liver, peribronchiolar regions of lung). Findings suggest localization of IL-2R to the cell membrane & cell processes of the lymphocytes.

Comments:

- The Ab used only recognizes the p55 alpha subunit of the IL-2 receptor (the Tac antigen), which is expressed only on activated T cells. The p75 beta subunit and the p64 gamma subunit were not evaluated. Endothelial cells are able to bind IL-2 without the p55 subunit. In addition, the sponsor did not look at the presence of IL-2 receptor on endothelial cells, which appear to be predominantly involved in the clinical adverse events noted thus far.

- The human IL-2R is present in 3 forms: low, intermediate, and high affinity. The low affinity IL-2R is made up of a 55 kD (p55, Tac, α chain, CD25) protein (K_d = -10 nM) and is unable to mediate internalization of bound ligand or signal transduction. The 64 kD (p64, I' chain) protein and the 75 kD (p75, β chain, CD122) protein separately, are limited in ability to bind IL-2R, but when combined, β-I' heterodimer can bind IL-2R with intermediate affinity (K_d = -1 nM). Both β and I' chains are needed for the receptor to internalize ligand and mediate signal transduction.
The low affinity IL-2R is made up of p55 and the intermediate IL-2R of p75/p64.
Noncovalent complexing of all three chains results in a high affinity complex (K_d = -10 pM). Expression of this complex in humans is associated with immune activation.

- The p75/p64 complex alone or in combination with p55 internalizes bound IL-2. The high affinity complex is the biologically relevant form of the IL-2R on mature activated T cells, with expression generally restricted to activated T cells, B cells, and monocytes. When DAB_{389}IL-2 binds to all 3 forms of the IL-2R, cells that express only the low or intermediate affinity receptor are at least 100-fold less sensitive to the fusion protein. Once DAB_{389}IL-2 binds to the surface of the high affinity form of the IL-2R, the molecule is _____ and _____ causing ceil death.

4. Generation of an Active Metabolite of DAB_{389}IL-2 by Proteolytic Cleavage Between the Active and Membrane Associating Domains: A Summary Report

The diphtheria portion of the product consists of two domains - one is responsible for the enzymatic activity of the product and the other is responsible for membrane association. Cleavage of the ~ amino acid loop in fragment A connecting these domains is necessary for the killing of sensitive target cells.

The sponsor notes that sera levels of biologically active product are higher than sera levels of immunoactive product. The bioassay indicator cell line [C91/PL] is several-fold more sensitive to nicked product (limited proteolysis) than intact $DAB_{389}IL-2$ (i.e., potentiation of biological activity). Proteolytic cleavage of $DAB_{389}IL-2$ can occur during the blood clotting process, thus it is possible that the enhanced biological activity observed occurred with sample preparation or in vivo. Various studies were performed to address this issue.

Findings:

1) Rapid processing of blood samples in the presence of EDTA resulted in lower in vitro nicking, as sera samples showed -27% intact $DAB_{389}IL-2$, while plasma samples showed -71% intact protein.

2) In vivo nicking of DAB,,IL-2 occurred in naive rats in a time-dependent manner, with a half-life of -26 mins. Abs to the protein are likely to slow the process, but not prevent it.

3) $DAB_{389}IL-2$ is sensitive to proteolytic cleavage between the enzymatic & membrane-associating domains of the diphtheria toxin part of the molecule. The bioassay is more sensitive to the proteolytic molecule than the intact molecule - resulting in increased amounts of bioactive material in sera samples [in vitro bioactivity of the nicked material was -70% higher than the starting $DAB_{389}IL-2$]. Thus the ELISA values are a more representative PK profile.

4) Fully nicked protein was not as well tolerated by rats as was the intact protein. Rats IV injected with nicked protein at 25 μg/kg/day for 10 days displayed more severe clinical signs (thin, rough haircoat) compared to rats dosed with intact protein. Rats dosed with nicked protein at 75 μg/kg/day for 10 days died early or showed a poor physical appearance. This pattern was noted in all adverse effects exhibited: ↓ BWs; ↑ BUN, creatinine, bilirubin, ALT, AST, LDH; ↓ albumin, protein, globulin, calcium; acute renal tubular necrosis; and hepatic inflammation.

5) The survival of mice bearing CP3 murine tumor cells that were IV injected with nicked DAB protein at 30 μg/kg/day for 5 or 10 days was slightly lower compared to mice injected with intact protein.

Comment:

- Note that in vivo, the intact protein was converted to nicked material at about the same rate that it was cleared from the circulation (half-life of -24 min).

5. In Vitro Studies Comparing the Mechanism of Action of Native $DAB_{389}IL-2$ and Trypein-Nicked $DAB_{389}IL-2$ on Tumor Cells and on Activated Normal Lymphocytes

The intent of the studies was to determine whether limited proteolysis [i.e., nicking] in vitro, also potentiated the biological activity of the DAB protein:

1) High affinity human IL-2R-expressing tumor cells (C91/PL, HUT 102/6TG) -incubated with intact or nicked $DAB_{389}IL-2$, resulted in higher biological activity of the nicked protein (2.3 to 28-fold) for the C91/PL line only [via the assessment of ^{3}H-leucine uptake].

2) Human PBMCs and murine spienocytes that were activated with OKT3 and incubated with intact or nicked $DAB_{389}IL-2$ showed a slight increase in biological activity (1.3-3.1-fold) for the nicked material on the PBMCs.

3) Both nicked and intact protein were able to compete in a comparable manner with radiolabeled IL-2 for binding to the high affinity IL-2R.

4) C91/PL cells incubated with intact and nicked protein for different time intervals, showed a dose-dependent response in the contact time needed to induce 50% inhibition of protein synthesis for both species. The times required to achieve IC,, were shorter for the nicked protein. The time needed to reach the IC_{50} for the nicked material ranged from 0.4-0.7 hr at 10^{-8} M to 1.3-1.4 hrs at 10^{-10} M of $DAB_{389}IL-2$.

Proteolytfc nicking of the DAB protein may shorten IL-2R contact time and enhance intracellular processing of $DAB_{389}IL-2$ by sensitive cells.

6. In Vitro Studies on the Interaction of $DAB_{389}IL-2$ with Human CD4+ Lymphocytes and CD8+ Lymphocytes

The intent of this study was to determine whether any differences in the ability of $DAB_{389}IL-2$ to affect inhibition of protein synthesis in activated CD4+ and CD8+ lymphocytes exist [due to any differences in the composition or level of IL-2R expression].

Human PBMCs were isolated and CD4+ and CD8+ subpopulations separated. Incubation of each subpopulation with PHA resulted in a progressively increased capacity to synthesize DNA [increased uptake of ^{3}H-thymidine]. Incorporation of ^{14}C-leucine began to decline by 48 hrs. There were no major differences between the subpopulations. An apparent increase in CD25 expression correlated with increased sensitivity of CD4+ and CD8+ cells to the fusion protein - median IC_{50} of 114.3 pM (activated CD4+, with 96% expressing CD25) vs. 67.6 pM (activated CD8+, with 86%

expressing CD25) at 24 hrs. The IC,, changed to 5.8 pM (CD4+) and 6.8 pM (CD8+) at 48 hrs, and to 5.8 pM (CD4+) and 3.8 pM (CD8+) at 72 hrs. The sponsor concludes that there was no difference in PHA-activated CD4+ and CD8+ cells.

7. Investigation into the Potential for Interaction of [^{35}S]DAB_{389}IL-2 with a High Molecular Weight Entity in Serum

The sponsor explored the possibility that the product'-could associate with plasma factors that could affect circulating DAB_{389}IL-2 levels.

Fresh plasma/sera from SD ♀ rats (naive & preimmune) and humans (with detectable & nondetectable titers) were spiked with 1 μg of [^{35}S]DAB_{389}IL-2 at 37°C for 15 mins, followed by size exclusion chromatography ——— to determine the approximate MW of radioactive fractions. The main radioactive peak eluting represented the monomer (MW of -60 kD). Varying amounts of radioactivity (2-40%) eluted at a time consistent with very high MW material (>350 kD). The amount of mean high MW radioactive material found in samples with Ab titers was >78% [possibly association with the Ab?], thus the amount of monomer was notably less [0-17%]. This high MW radioactive material was not detected in samples incubated with excess cold DAB_{389}IL-2 & radioactive DAB_{389}IL-2, nor was it detected when samples were run under denaturing conditions - potentially indicating that the association of [^{35}S]DAB_{389}IL-2 with the high MW material is non-covalent.

8. In Vitro Studies *on* the Mechanism of DAB_{389}IL-2 Induced Cell Death

Human PBMCs cultured with OKT3 for 48 hrs displayed maximal CD25 receptor expression and high blast transformation (-40% of the cells), thus 48-hr activated T cells were used in the cell death studies. The LDH (lactate dehydrogenase) assay was used - which measures the amount of LDH released from the cytosol of damaged cells into the culture supernatant - thus reflected the necrotic cells. Apoptosis was measured by a quantitative flow cytometric assay based on dUTP staining of DNA fragments. Cells were incubated with DAB_{389}IL-2 (0.04-10,000 pM).

The percent of CD25-bearing activated T cells dying by necrosis in cultures exposed to 10 nM of DAB_{389}IL-2 was the highest, at 9%. Only 2-16% of the untreated control activated cells were positive for dUTP compared to 30-74% of the cells exposed to DAB_{389}IL-2 (dose-related manner). In addition, a 48 hr exposure time did not increase the numbers of apoptotic cells.

9. Stability of DAB_{389}IL-2 in Human Serum and Rat Serum and Plasma

DAB_{389}IL-2 levels of 0.188, 0.938, or 9.38 μg/5 mL were added to whole rat or human blood, followed by storage of the isolated serum/plasma at -20 ±5°C, and tested for bioactivity (every 2 months) and immunoreactivity (every 6 months) for one year. The DAB_{389}IL-2 in the frozen specimens was reduced at each analysis: the bioactivity had a half-life of only about 15 months (maximum deterioration of -20% with 6 months storage) and the immunoreactivity exhibited a mean half-life of 3 years in human samples.

10. Evaluation of DAB_{389}IL-2 Cytotoxicity and IL-2R Expression in a Panel of Primary Human Cell Types

Cryopreserved normal human cells were cultured overnight with 50 μL of DAB_{389}IL-2 (0.3-576 ng/mL; 5 x 10^{-12} to 5 x 10^{-8}M), followed by culture with 50 μL of 1.5 μCi ^{14}Leucine and counting of radiolabel. [IC_{50} = amount of DAB_{389}IL-2 needed to reduce protein synthesis by 50%]

Levels up to 576 ng/mL did not inhibit protein synthesis in any of the cell types. None of the cell types expressed the p55 chain of IL-2R and only a few (shown below) expressed low levels of the p75 chain [determined by RT-PCR analysis for mRNA levels]:

Table 3-1

Sensitivity of Human Primary Cultures

Cell Type	IL-2R Status* p55	p75	IC_{50} (ng/mL)
proximal tubular epithelial cells		•	>576
renal cortical epithelial cells	•	•	>576
renal mesangial cells	•	low	>576
dermal microvascular endothelial cells	•	•	>576
pulmonary may endothelial cells			>576
pulmonary artery smooth muscle	•		>576
skeletal muscle cells		low	>576
epidermal keratinocytes		low	>576
dermal fibroblasts			>576
bronchial/tracheal epithelial cells			>576

• = negative: < 10^5 copies mRNA/50 ng total RNA ; low: < 5 x 10^5 copies; moderate: 5 x 10^5 to 5 x 10^6 copies; high: > 5 x 10^6 copies

11. Characterization of the Specificity, Kinetics, and Mechanism of DAB_{389}IL-2 Cytotoxicity and Correlation with IL-2 Receptor Expression in a Panel of Tumor Cell Lines

One Gibbon ape and 13 human tumor cryopreserved cell lines were cultured overnight with 50/100 μL of DAB_{389}IL-2 (0.3-576 ng/mL; 5 x 10^{-13} to 5 x 10^{-8}M), followed by culture with 50 μL of 1.5 μCi ^{14}Leucine and counting of radiolabel. [IC_{50} = amount of DAB_{389}IL-2 needed to reduce protein synthesis by 50%]

The amount of $DAB_{389}IL-2$ needed to achieve $IC_{,,}$ ranged from 0.2-11.5 ng/mL (10^{-12} to 10^{-10}M) . Generally, tumors that were sensitive to protein inhibition expressed mRNA for the p64, p55, and p75 subunits of IL-2R. This inhibition led to cell death. Tumors that were insensitive to the cytotoxic action of $DAB_{389}IL-2$ generally expressed undetectable/ low mRNA levels for p55/p75:

Table 3.1-I

Tumor Cell Lines **Sensitive** to **DAB_{389}IL-2-Mediated** Cytotoxicity

cell Line	Type	IC_{50} (ng/mL)	IL-2R mRNA Expression p55	p75
Hut 102/6TG	cutaneous T-cell lymphoma	0.3	high	moderate/high
C91/PL	HTLV-I transformed T-cell	0.5	high	moderate
MT-2	HTLV-I transformed T-cell	115	high	high
HH	cutaneous T-cdl lymphoma	5.5	low	high
C8215	T-cell leukemia	5.8	moderate/high	moderate

Table 3.1-2

Tumor Cell Lines Insensitive to **DAB_{389}IL-2-Mediated** Cytotoxicity

Cell Line	Type	IC_{50} (ng/mL)	IL-2R mRNA Expression p55	p75
Hut 78	cutaneous T-cell lymphoma	>576	moderate	moderate
MT-1	adult T-cell leukemia	>576	high	low
MLA-144	T-cell lymphoma	>576	moderate	low
YT 2C2	acute lymphoblastic leukemia	>576	low	high
SKW 6.4	B cell	432	low	moderate
u937	large cdl lymphomr	>576	negative	low
Daudi	Burkitt lymphoma	>576	low	negative
SW684	fibrosarcoma	>576	negative	negative
NCI-H929	plasmacytoma	>576	negative	negative

The addition of excess IL-2 reduced/blocked the cytotoxic action of $DAB_{389}IL-2$ for the high affinity (p55+, p75+, p64+) IL-2R expressing tumors:

Table 3.2-1

Specificity of **DAB_{389}IL-2-Mediated** Inhibition of **Protein** Synthesis

Cell Line	Type	IC_{50} (ng/mL)	+ IL-2 IC_{50} (ng/mL)	+ IL-4 IC_{50} (ng/mL)
Hut 102/6TG	cutaneous T-cell lymphoma	0.3	17	0.3
C91/PL	HTLV-I transformed T-cell	0.5	>58	0.6
MT-2	HTLV-I transformed T-cell	115	>58	11.5
HH	cutaneous T-cell lymphoma	5.5	>58	5.8
C8215	T-cell leukemia	5.8	346	5.8

The time needed for $DAB_{,,}$IL-2 to be in culture with tumor cells in order to obtain 50% protein synthesis was dependent on concentration. Times ranged from ≤1 hr to > 6 hrs and did not appear to be dependent on tumor type. Human tumor cells exposed to 50 or 500 ng/mL (10^{-9} to 10^{-8}M) of $DAB_{389}IL-2$ for ≤35 mins generally resulted in 50% inhibition of protein synthesis within 4 hrs.

Data also indicate that inhibition of cellular protein synthesis for $DAB_{389}IL-2$ is similar to that for diphtheria toxin - mediated by ADP-ribosylation of elongation factor-2 [after translocation into the cytosol, the DT catalyzes the **cleavage** of NAD and the covalent linkage of ADP-ribose to elongation factor-2, causing protein inhibition].

12. Effect of $DAB_{389}IL-2$ After 20-Hour Exposure in Human Liver and Kidney Slices

Human liver and kidney slices from a male and a female donor were obtained [from the _____] were preincubated in media for 1 hr (at 37°C), followed by incubation with $DAB_{389}IL-2$ (up to 10 nM) for 20 hrs, then incubated (4 hrs) with ^{3}H-leucine. After appropriate homogenization/disruption of the slices, the radiolabel amount was quantitated. No statistically significant effects on protein synthesis in either tissue were noted.

13. In Vitro Inhibition of Protein Synthesis by $DAB_{389}IL-2$ in Primary Cultures of Rat Hepatocytes, Kupffer Cells, and Renal Proximal Tubule Epithelial Cells

Hepatocytes, Kupffer cells, and renal proximal tubule epithelial cells [RPTE] were isolated from ♀ SD rats and incubated with 1 x 10^{-11} to 2 x 10^{-7}M of $DAB_{389}IL-2$ for 24 hrs, followed by exposure to ^{14}C-leucine for 4 hrs for assessment of protein synthesis. In addition, some incubation media contained 10^{-6}M IL-2 as a competitive inhibitor for IL-2R binding for 2-8 hrs.

The $IC_{,,}$ of $DAB_{389}IL-2$ was 3 x 10^{-8}M for hepatocytes; 5 x 10% for Kupffer cells; and >1 x 10^{-7}M for RPTE cells. The additional presence of IL-2 did not affect the IC_{50}s - thus the process of inhibition of protein synthesis is not controlled by IL-2R, but possibly due to some other nonspecific endocytosis mechanism.

Comment:

- A bolus dose of 25 μg/kg in naive rats = mean sera levels of 7 x 10^{-9}M (402 ng/mL; study #3 in the PK/ADME section) - the $IC_{,,}$ for inhibition of protein synthesis in human tumor cells (study #ii).

14. Evaluation of the Impact of DAB,,,IL-2 on Primary Cultures of Human Endothelial Cells

Human endothelial cell types [dermal microvascular, lung microvascular, umbilical vein, pulmonary artery] were exposed to $DAB_{389}IL\text{-}2$, ——————— or ——————— at 10^{-7} to $10^{-11}M$ for 18-24 hrs, followed by incubation with ^{14}C-leucine for 2 hrs to evaluate protein synthesis. The cells were also incubated with the different DAB proteins and observed for morphological changes, as well as cytokine production [IL-6, TNFα, IL-la].

The human dermal and lung cells and umbilical vein cells were insensitive to $DAB_{389}IL\text{-}2$ cytotoxicity, while pulmonary artery cells showed an IC,, of 5 x $10^{-8}M$ (2882 ng/mL). Morphologic changes were observed for the cells with high concentrations of all material used [thus were not probably not related to protein synthesis]. IL-6 levels increased with time at $DAB_{389}IL\text{-}2$ levels of $\leq 10^{-8}M$ (576 ng/mL), but was inhibited at $10^{-7}M$ (5765 ng/mL).

Comments:

- Human dermal cells and pulmonary artery cells do not express mRNA for the p55 or the p75 subunit of the IL-2R.

- IL-2 is associated with increased capillary permeability - edema, ascites, pleural effusions, interstitial edema, hypotension, and oliguria. Rats given $DAB_{389}IL\text{-}2$ have displayed inflammation in the lungs and kidneys, as well as perivascular edema and vascular congestion in the lungs (study #95163, 96131) at doses 210 μg/kg/day for 4 weeks.

Pseudomonas exotoxin and ricin A-containing immunotoxins also have caused vascular effects on endothelial cells.

15. IL-2R Contact Time Studies with Activated Human CD8+ and CD4+ Lymphocytes

Normal human PBMCs were isolated and activated with OKT3 (anti-CD3) Ab for 72 hrs. CD4+ and CD8+ cells were selected and purified and quantitated (via FACS), followed by incubation with 0.01 to 10 nM $DAB_{389}IL\text{-}2$ for 18 hrs. Cells were then exposed to ^{14}C-leucine - **Protein Synthesis Inhibition.**

Activated human PBMCs were incubated with 10 pM to 1 nM $DAB_{389}IL\text{-}2$ for from 0.5-300 mins, followed by interruption of the fusion protein:cellular IL-2R interaction (with IL-2) and addition of ^{14}C-leucine - **Contact Time.**

Findings:

Purity = 297% [CD8+], 295% [CD4+]
CD25+ expression = 83% [CD8+], 81% [CD4+]
Viability = >97%
CT_{50} [exposure level resulting in 50% reduction in protein synthesis] = 5 min [CD8+], 11 min [CD4+] - for 100 pM $DAB_{389}IL\text{-}2$

$CT_{,,}$ = 114 min [CD8+], 84 min [CD4+] - for 10 pM $DAB_{389}IL-2$

Conclusion: OKT3-activated human CD8+ and CD4+ cells show similar $CT_{,,}$ values at the concentrations of $DAB_{389}IL-2$ evaluated.

16. Assay Development and Performance Report: ELISAs for the Determination of Anti-DAB&L-2, Anti-Diphtheria Toxin, and Anti-IL-2 Ab Titers in Human, Monkey, Rat, and Mouse Serum

This report documents the method and performance of a series of 12 semi-quantitative Ab ELISAs intended to measure the titer of Abs reacting to $DAB_{,,}$ IL-2, DT, or IL-2 in the sera of various animal species. For further details, refer to this study report.

17. Assay Development and Performance Report: Determination of $DAB_{389}IL-2$ Levels by ELISA with Anti-fragment B Capture and Chemiluminescent Detection

This report documents the method and performance of the assay used for the determination of $DAB_{389}IL-2$ levels in preclinical and clinical sera by ELISA. For further details, refer to this study report.

18. Assay Development and Performance Report: Determination of $DAB_{389}IL-2$ Bioactivity Levels by Bioassay

This report shows that high concentrations (up to 2500 pM) of sIL-2Rα [soluble α chain (Tac) of the IL-2R] do not interfere with the detection of citrate-formulated $DAB_{389}IL-2$ in the bioassay. For further details, refer to this study report.

19. Assay Validation Report: Determination of $DAB_{389}IL-2$ Bioactivity Levels in Preclinical and Clinical Serum Samples by Bioassay

This report documents the validation of the bioassay, SOP ------- — for determination of $DAB_{389}IL-2$ bioactive levels in preclinical and clinical sera. For further details, refer to this study report.

20. Assay Development and Performance Report: Determination of Neutralizing Anti-DAB_{389}IL-2 Antibody Levels

This report documents the validation of the neutralizing assay - intended to determine neutralizing anti-DAB_{389}IL-2 Ab levels. This assay is based on the measurement of diphtheria toxigenicity and serum antitoxin levels. The assay uses a_____ _____ The presence of neutralizing Abs will block the interaction of DAB_{389}IL-2 with the IL-2R, thus reducing the bioactivity. For further details, refer to this study report.

21. Measurement of DAB_{389}IL-2 Blood Levels

This report documents the relationship between the ELISA, measuring DAB_{389}IL-2 levels, and the assays used to measure the immunogenicity of the protein - 1) an ELISA to measure total Abs to DAB_{389}IL-2 and 2) a tissue culture-based assay to measure the Abs that neutralize the in vitro bioactivity of the protein. For further details, refer to this study report.

In vivo

1. Impact of Treatment with DAB_{389}IL-2 on Preimmune and Naive C57BL/6 Mice in the CP3 Model of IL-2R Expressing Malignancy

Methods: C57BL/6 ♀ mice were preimmunized with 20 μg/animal of diphtheria toxoid (SC, for 4 days), followed two weeks later by blood collection and determination of Abs that cross-react with DAB_{389}IL-2. Preimmune and naive mice were IV injected with 1×10^6 CP3 cells, followed by IV injection of 30 or 60 kg/kg/day of DAB_{389}IL-2 for 10 days. All survivors were killed on day 95.

Findings:
Mean survival times for controls were 32 days (preimmune) and 38 days (naive). Mean survival times in treated mice were 92 days (naive, 30 μg/kg - only one death); 52 days (preimmune, 30 μg/kg); and 62 days (preimmune, 60 μg/kg). Weight loss was noted in the naive mice only.

The presence of circulating anti-diphtheria toxoid Abs did not affect onset of disease. The DAB_{389}IL-2 prolonged the survival of both naive & preimmune mice. Survival times were longer in naive mice, indicative that some inhibition of DAB_{389}IL-2 activity occurred in the preimmune mice.

Comments:
- [Per the sponsor] In vitro sensitivity (IC_{50}) of CP3 cells to DAB_{389}IL-2 is normally <0.5 ng/mL.

● [Per the sponsor] Malignant cells of certain leukemias and lymphomas express IL-2R. The CP3 murine tumor cell line expresses the p55/p75/p64 receptors. The CP3 model of murine lymphoma [in C57Bl/6 mice] has been used to assess the efficacy of various doses and dosing regimens for $DAB_{389}IL-2$. Tumors develop in 80-100% of control mice injected with 10^6 CP3 cells. The model is reflective of the late stages of human lymphoma, as tumors are distributed throughout the lymphatic system and end up metastasizing.

2. Impact of Dose and Schedule of $DAB_{389}IL-2$ in a Murine Model of IL-2 Receptor Expressing Malignancy: A Summary Report

Methods: C57BL/6 ♀ mice were' IV injected with 1 x 10^6 CP3 cells (day 0), followed by IV injections of 0, 7, 15, 20, 30, 40, or 60 µg/kg/day, initiating on day 1, 2, 6, or 10, and continuing for 3, 5, 7, or 10 days. Other regimens included episodic dosing of 3 or 5 doses/week for 2-4 wks. The study terminated on -day 90.

Findings:
The mean survival time of untreated mice was 30-38 days. DAB,,, IL-2 doses of 7-40 µg/kg/day resulted in significant increases in survival, as well as delaying or preventing the onset of clinical signs noted in the untreated mice [i.e., enlarged LNs, etc...]. The most effective dosing regimen was daily injection on days l-10, followed by injection on days l-7. Increased survival times were also seen when dosing was started as late as day 3 or day 6, but no efficacy was noted when dosing started on day 10. Note that BW losses were noted in a dose-related manner at all doses of $DAB_{389}IL-2$ when injected daily for 10 days.

3. Specificity of $DAB_{389}IL-2$, ———— and --------, in Murine Models of IL-2R Expressing and IL-2R Negative Malignancy
On day 0, ♀ C57Bl/6 or DBA/2 mice were IV injected with either EL-4 [murine lymphoma] or P388 [leukemia of DBA/2 mice]. Both of these tumors have been shown to be insensitive to $DAB_{389}IL-2$ in vitro [IC_{50} of 2 x 10^{-7}M (EL-4) and >7 x 10^{-8}M (P383)]. Mice were IV injected with 0, 30, or 40 µg/kg/day for days l-10 of each DAB analog.

Findings:
Neither of the functionally inactive analogs - ———— nor ———— was effective in the murine tumor models, with survival times of 17 days in EL-4 mice compared to 65 days with $DAB_{389}IL-2$. None of the three molecules were effective in the P388 models [deaths by day 161.

Comment:
● The sponsor postulates that the reason for cytoxicity of the EL-4 cell line may be the in vivo binding of the endogenous β and I' portions of the IL-2R chain expressed by the EL-4 cells, resulting in internalization of $DAB_{389}IL-2$.

4. Adoptive Transfer of Lymph Node Cells from CP3-Injected Mice: Evaluation of Subsequent Tumor Development and Sensitivity to $DAB_{389}IL-2$
C57CL/6 ♀ mice injected with from 10^2-10^6 CP3 tumor cells/mouse, showed that a threshold of $\geq 10^4$ CP3 cells are needed to cause the clinically apparent disease - with death in 80-100% of the mice.

Note that in other efficacy studies performed, a dose of 10^6 CP3 cells was used to establish the tumor model.

Other Studies

1. Impact of $DAB_{389}IL-2$ in a Murine Model of IL-2R-Expressing Malignancy: A Summary Report
Species: C57BL/6 ♀ mice
Methods: Mice were IV injected with 10^6 CP3 tumor cells (expresses high affinity IL-2R, which results in tumors in 80-100% of animals injected), followed by various dosing schedules for $DAB_{389}IL-2$.

Findings:
Conclusions of note included:

- Once daily 10-day dosing resulted in extended survival of mice. Comparison to other treatment regimens found that once daily dosing at 44 $\mu g/kg/d$ resulted in extended survival.
- $DAB_{389}IL-2$ had no effect on survival of mice with IL-2R negative EL4 tumors.
- Delayed treatment, beginning on day 6, was efficacious.
- Pre-existing antibodies to the DT portion of the molecule did not impact survival time of the $DAB_{389}IL-2$ treated mice.

PK/ADME Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. Partition of ^{35}S-DAB$_{389}$IL-2 in Whole Rat and Human Blood; report #94165; performed at Seragen (non-GLP); lot [redacted] 8/94; vol 12

2. Antibody Levels and PK Analysis of Serum Concentrations from Cynomolgus Monkeys receiving DAB,,, IL-2 IV for Up to 30 Days; report #95117 [data from #95107]; performed at Seragen (non-GLP); 2/96; vol 14

3. PK Analysis of DAB,,, IL-2 Serum Concentrations in Naive and Preimmune Rats: A Summary Report; report #95118; performed at Seragen (non-GLP); lot [redacted]; 3/96; vol 14

4. Biodistribution and Excretion of [$_{35}$S]DAB$_{389}$IL-2 in Naive and Preimmune SD Rats; report #96026; performed at Seragen (non-GLP); lot [redacted] 6/96; vol 16

5. PK of DAB$_{389}$IL-2 in Partially Hepatectomized and Unilaterally Nephrectomized SD Rats; report #96044; performed at Seragen (non-GLP); lot [redacted] 6/96; vol 16

Reports Not Included in the BLA (from IND [redacted])
1. Clearance, Biodistribution, and Excretion of DAB$_{389}$IL-2 in Rats: A Summary Report; report #94020; performed at Seragen (non-GLP)

PK/ADME Studies

1. Partition of ^{35}S-DAB$_{389}$IL-2 in Whole Rat and Human Blood
Six weeks prior to study start, 4/8 SD rats were immunized with diphtheria toxin. Blood collected from the naive and preimmune rats [supported by detectable anti-DAB$_{389}$IL-2 Ab titers1 was spiked with 2 μg of ^{35}S-DAB$_{389}$ IL-2 and the radiolabel measured in sera and plasma. This procedure was also performed with blood obtained from two human with detectable Ab titers and from two humans with nondetectable titers.

Findings:
Recovery data show that DAB,,, IL-2 does not appear to associate with the cellular component of normal rat and human blood. The labeled protein was found in the serous fraction, regardless of the presence of detectable Ab titers.

2. Antibody Levels and PK Analysis of Serum Concentrations from **Cynomolgus Monkeys receiving DAB_{389}IL-2 IV for Up to 30 Days**
[data from #95107]
Species: cyno monkeys (2-3/sex/grp), with nondetectable/low anti-DAB_{389}IL-2 Abs & anti-IL-2 Abs
Dose Levels: 0, 2.5, 10, 25 µg/kg/day
Route/Duration: IV/variable regimens

Findings:
Abs - **2.5, 10, 25 µg/kg/day x 30 days**
Day 28 - anti-DAB - 87% positive (moderate -high titers)
Day 28 - anti-DAB [neutralizing] - positive (low-high titers)
Day 28 - anti-IL-2 - positive (low titers)

25 µg/kg/day x 15 days x 2 courses
Day 43 - anti-DA5 - 100% positive (high titers)
Day 43 - anti-DAB [neutralizing] - unable to evaluate due to inadequate sample
Day 43 - anti-IL-2 - positive (high titers)

The presence of the anti-DAB,,,IL-2 Abs correlated to the general cessation of weight loss. One grp 4A monk that did not exhibit an Ab response died on day 27. Monks in grp 4C gained weight during days 30-44 - the second cycle of dosing - and transaminase levels lowered to near baseline levels in this dosing period [but were starting to rise on terminal day 441.

PK - Dav 1 - DAB_{389}IL-2
Correlation between bioassay & ELISA = 0.84
$t_{½}$ = 41 ±17 min (bioassay) vs. 35 ± **15** min (ELISA)
Vd = 33 ±67 mL/kg (bioassay) vs. 59 ± **31** mL/kg (ELISA)
Cl = 0.39 ±0.41 mL/kg (bioassay) vs. 1.30 ± 0.91 mL/kg (ELISA)

Day 29 - DAB_{389}IL-2
Sera levels (bioassay) were ND/low - due to Ab blockage of the detection/T clearance rate of molecule
1) levels were detected in sera with ——— but 2) the immuno-reactive levels were ↓ compared to day 1 [↑ clearance ?]

Comments:

The bioassay is more sensitive to the proteolytic molecule than the intact molecule - resulting in increased amounts of bioactive material in sera samples. Thus the ELISA values are a more representative PK profile.

● Two minutes of exposure of IL-2R expressing cells to 0.1 μg/mL of $DAB_{389}IL-2$ results in 50% maximal inhibition of protein synthesis. In this study, the peak sera levels (C_{max}) ranged from 0.025-0.570 μg/mL.

3. PK Analysis of $DAB_{389}IL-2$ Serum Concentrations in Naive and Preimmune Rats: A Summary Report

Methods: SD ♀ rats were preimmunized with 100-144 μg/animal of diphtheria toxoid (SC, for 4 days), followed two weeks later by blood collection and determination of Abs that cross-react with $DAB_{389}IL-2$. Rats, designated with low or high Ab titers and [along with naive rats], were given a single IV injection of 25 or 75 μg/kg ^{35}S-$DAB_{389}IL-2$ or 25 μg/kg $DAB_{389}IL-2$. Sera samples were analyzed by bioassay, ELISA, and radiolabel counting.

Findings:

PK data are listed below:

Table 3.1-3

Pharmacokinetic Parameters - 25 μg/kg Dose Level
(Mean ± Standard Deviation)

Bioassay

Test Group	C_0 (kU/mL)	V_c (mL/kg)	$t_{1/2}$ (min)	AUC (kU/kg x min)	Cl (mL/kg x min)
Naive	83 ± 14	16 ± 3	22 ± 4	2581 ± 365	0.51 ± 0.08
Low Antibody	38 ±16	46 ± 38	20 ± 10	1077 ± 663	1.99 ± 1.72
High Antibody	6 ± 1	223 ± 29	22 ± 6	170 ± 98	9.02 ± 5.17

Product ELISA

Test Group	C_{max} (ng/mL)	V_c (mL/kg)	$t_{1/2}$ (min)	AUC (ng/kg x min)	Cl (mL/kg x min)
Naive					
Low Antibody	402 ± 3	67 ±	26 ±	18859 ± 3	1.91 ±
High Antibody	257'54	100 ± 21	14 ± 7	4236 ±1801	6.49 ± 2.76

Radioactivity

Test Group	C_{max} (cpm·10⁻³/mL)	V_c (mL/kg)	$t_{1/2}$ (min)	AUC (cpm·10⁻³/kg x min)	Cl (mL/kg x min)
Naive	164 ± 26	50 ± 7	3 1 ±10	8301 ± 1987	1.03 ± 0.30
Low Antibody	120 ± 36	76 ± 34	41 ± 33	6629 ± 1817	1.35 ± 0.55
High Antibody	265 ± 27,	82 ± 41	45 ± 14	4136 ± 370	4.07 ±3.14

Table 3.1-4

Pharmacokinetic Parameters - 75 μg/kg Dose Level
(Mean ± Standard Deviation)

Bioassay

Test Group	C_0 (kU/mL)	V_c (mL/kg)	$t_{1/2}$ (min)	AUC (kU/kg x min)	Cl (mL/kg x min)
Naive	226 ± 79	19 ± 8	28 ± 6	8168 ± 2708	0.51 ± 0.15
Low Antibody	192 ± 28	20 ± 3	25 ± 4	6411 ± 1123	0.6, ± 0.10
High Antibody	72 ± 41	113 ± 142	12 ●	1118 ± 665	7.23 ± 8.91

Product ELISA

Test Group	C_{max} (ng/mL)	V_c (mL/kg)	$t_{1/2}$ (min)	AUC (ng/kg x min)	Cl (mL/kg x min)
Low Antibody	1537 ± 341	54 ± 5	23 ± 3	46013 ± 821	±
High Antibody	1098 ± 250	70 ± 14	15 ± 4	239165726	3 14 ± 0.09

Radioactivity

Test Group	C_{max} (cpm·10⁻³/mL)	V_c (mL/kg)	$t_{1/2}$ (min)	AUC (cpm·10⁻³/kg x min)	Cl (mL/kg x min)
Naive	550 ± 100	46 ± 8	31 ± 4	25.68 ± 6207	1.0) ± 0.25
Low Antibody	599 ± 102	42 ± 8	29 ± 6	25712 ± 5271	0.99 ± 0.25
High Antibody	695 ± 213	38 ± 15	147 ± 128	100866 ± 144698	1 19 ± 0.76

Sera levels of the product were lower for preimmune rats compared to naive animals, possibly due to some type of blocking in the detection of bioactive product or from an increased clearance of the fusion toxin. These results were noted via bioassay, ELISA, and radiolabel analysis.

Comment:

● [Per the sponsor] Since the human IL-2 part of the product is foreign to rats, diphtheria toxoid rather than $DAB_{389}IL-2$ was used as the immunogen in order to more closely mimic the type of Abs observed in humans.

4. Biodistribution and Excretion of [^{35}S]DAB$_{389}$IL-2 in Naive and Preimmune SD Rats

Methods: SD Q rats were preimmunized with 100 μg/animal of diphtheria toxoid (SC, for 4 days), followed three weeks later by IV injection of [^{35}S]DAB$_{389}$IL-2. Tissue, blood, and urine samples were taken at 0.25, 1, 4, 24, and 48 hrs and analyzed by radiolabel counting. Sera samples were analyzed by bioassay and ELISA.

Findings:
Radiolabel - % of total dose

Naive ♀s

15 mins - plasma (62%), liver (13%), kidney (4%), lungs (2%), spleen (2%)

1 hr - plasma (13%), liver (11%), kidney (2%), lungs (2%), spleen (1%), pancreas (4%), GI tissues (2%)

4 hr - plasma (8%), liver (10%), kidney (2%), lungs (1%), spleen (1%), pancreas (4%), GI tissues (2%)

24 hrs - plasma (6%), liver (10%), kidney (2%), lungs (1%), spleen (1%), pancreas (2%), GI tissues (1%)

48 hrs - plasma (4%), liver (5%), kidney (1%), lungs (1%), spleen (1%), pancreas (1%), GI tissues (1%)

Preimmune ♀s

15 mins - plasma (50%), liver (20%), kidney (3%), lungs (2%), spleen (1%)

1 hr - plasma (9%), liver (14%), kidney (1%), lungs (1%), spleen (1%), pancreas (3%), GI tissues (2%)

4 hr - plasma (11%), liver (12%), kidney (2%), lungs (1%), spleen (1%), pancreas (4%), GI tissues (2%)

24 hrs - plasma (6%), liver (7%), kidney (2%), lungs (1%), spleen (1%), pancreas (1%), GI tissues (2%)

48 hrs - plasma (4%), liver (7%), kidney (1%), lungs (1%), spleen (1%), pancreas (1%), GI tissues (1%)

For all Qs, about 20% of the radiolabel as excreted in the feces and urine. Two preimmune ♀s killed at 15 mins, displayed an accumulation (-20%) of silver grains in the kidney proximal tubules. A larger proportion of the radiolabel was found in the liver of preimmune rats at 15 mins only.

The tissue distribution pattern was similar for the naive and preimmune ♂s. Generally, all tissues had increasing T/P ratios with time, indicative of an accumulation of ^{35}S-material. Higher levels were noted in BM and esophagus, possibly reflective of some binding of DAB,,, IL-2 occurring in these tissues. Although -5% of the total radiolabel was seen in the plasma by 48 hrs, very little bioactive material was noted. Likely the parent DAB product is being metabolized and the amino acids excreted in the bile, feces, and urine or used in the tissues.

5. **PK** of **DAB$_{389}$IL-2** in Partially **Hepatectomized and Unilaterally** Nephrectomized SD **Rats**
Normal ♀ SD rats were partially (70%) hepatectomized or unilaterally nephrectomized were IV injected with 25 μg/kg DAB$_{389}$IL-2, followed by blood sampling and evaluation of sera via ELISA [lower limit of detection of 0.2 ng/mL] and via bioassay [inhibition of protein synthesis in a human IL-2R expressing cell line - lower limit of detection of -1 ng/mL]].

Findings:
Data are presented:

Table 3.2-1
Mean [redacted] Parameters - Product ELISA

Treatment	C_{max} (ng/ml)	V_d (ml/kg)	$t_{1/2}$ (min)	AUC (ng/ml•min)	Cl_T (ml/kg•min)
Normal	710 ± 168	37 ± 8	26 ± 3	18428 ± 5906	1.46 ± 0.42
Nephrectomized	508 ± 58	50 ± 5	29 ± 6	17791 ± 9809	1.66 ± 0.61
Hepatectomized	585 ± 152	45 ± 11	42 ± 9*	37451 ± 7590*	[illegible] • 0.15*

* p≤0.05 compared to normal

Table 3.2-2
Mean Pharmacokinetic Parameters - Bioassay

Treatment	C_{max} (U/ml)	V_d (ml/kg)	$t_{1/2}$ (min)	AUC (U/ml•min)	Cl_T (ml/kg•min)
Normal	72395 ± 20282	10 ± 3	32 ± 9	3077038 ± 567362	0.23 ± 0.04
Nephrectomized	62794 ± 13998	11 ± 3	27 ± 13	2216330 ± 427579	0.32 ± 0.06*
Hepatectomized	59328 ± 23251	13 ± 5	42 ± 16	3240202 ± 995722	0.23 • [illegible]

* p≤0.05 compared to normal

Liver weights of the hepatectomized rats were comparable to normals. However, the clearance rate was slower, the half-life longer, and the AUC higher compared to normals. The PK profile of nephrectomized rats was similar to controls.

Other Studies

1. **Clearance, Biodistribution,** and **Excretion of DAB$_{389}$IL-2 in Rats: A Summary Report**
Species: SD ♀ rats
Dose/Route: 44 μg/kg/day; IV

Methods: Several studies used ^{35}S-labelled material; other used unlabelled material.

Findings: IV bolus - circulating half-life = 30-40 min
clearance rate = 0.3 mL/kg.min

There are no cumulative PK effects - **day 1 & day 5 clearance** rates were comparable.

The clearance profile of total protein (radioactive counts) differs from the profile noted for biologically active material. There was an initial rapid decrease in sera counts, followed by a plateau or slow rise in counts after 30 mins. Non-biologically active labelled material continued to circulate for at least 72 hrs postdose.
Very low levels of biological activity were found in sera samples from preimmune rats that received labelled material. The radiolabel remained in circulation for over 4 hrs. [The sponsor postulates that immune complexes **may** serve **as** a reservoir for the fusion toxin. It has not been determined as to whether the DAB_{389}IL-2 bound by antibody is biologically active, but the antibody **may** protect the molecule from degradation, thus preserving biological activity.]

The primary site of distribution of labelled material outside of the vasculature is the liver - at 15 mins postdose, 50% of the dose was found in the liver and 6% in the kidney. Urinary excretion was 15% of the injected dose over 72 hrs. **The liver is** probably **the primary site of metabolism - major toxicity is also noted here (↑ hepatic transaminases).**

Preclinical Toxicology Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. Toxicity of DAB_{389}IL-2 Administered IV for 10 **Days** in Naive and Preimmunized Mice: An Exploratory Study; report #94013; performed at Seragen (non GLP); lot .——— 4/94; vol 10

2. Toxicity, Clearance, & Efficacy of DAB_{389}IL-2 (——— Formulation vs. Citrate Formulation) in Rats: A Summary Report; report #94019; performed at Seragen (non GLP); lot ——— ——— & —— (citrate); 5/94; vol 10

3. Immunotoxicity of DAB_{389}IL-2 Administered — for 10 Days in Mice: A Summary Report; report #94020; performed at Seragen (non GLP); lot ——————— 7/94; vol 10

4. Comparative Toxicity Evaluation of DAB_{486}IL-2 vs. DAB_{389}IL-2 Administered IV to Mice; report #94066; performed at --——— ---- (non GLP) ; lot ------- (DAB_{389}) ; ——— (DAB_{486}) ; 6/94; vol 11

5. A Study to Compare the Clearance Pattern and Toxicity of Two Related Test Articles Following Administration by IV Injection to Cynomolgus Monkeys; report #94067; performed at [redacted] (per GLP); lot [redacted] (DAB_{389}); 1I18IB2 (DAB,,,); 8/92; vol 11

6. Effect of Diet on the Toxicity of DAB_{389}IL-2 Administered IV to Rabbits; report #94068; performed at [redacted] (per GLP); lot [redacted] 2/93; vol 12

7. A Comparison of DAB,, IL-2 Toxicity and PK in Male and Female Rats Following IV Bolus Administration: A Summary Report; report #95002; performed at Seragen (non-GLP); lot [redacted] 6/95; vol 12

8. Acute Toxicity of Citrate Formulated DAB_{389}IL-2 Following IP Injection in Mice and Guinea Pigs: Summary of Step-Down Studies; report #95012; performed at Seragen (non-GLP); lot [redacted] 3/95; vol 12

9. A Comparison of DAB,, IL-2 Toxicity and PK in Normal Mice and Mice with IL-2 Receptor Expressing Tumors: A Summary Report; report #95041; performed at Seragen (non-GLP); lot [redacted] 8/95; vol 12

10. A Comparison of the Toxicity and PK of DAB_{389}IL-2, [redacted] and [redacted] in Rats: A Summary Report; report #95093; performed at Seragen (non-GLP); lot [redacted] (DAB_{389}IL-2); 5B15CP2 [redacted] 5B06CP2 [redacted]; 2/96; vol 13

11. Toxicity of DAB_{389}, [redacted] and [redacted] Administered IV for 10 Days in Mice: A Comparison Study; report #94016; performed at Seragen (non-GLP); lot #unknown; located in IND [redacted], not included in this BLA

12. Evaluation of Toxicity and PK of DAB_{389}IL-2 in Cynomolgus Monkeys Following 30 Days of IV Administration; report #95107, performed at [redacted] (per GLP); lot [redacted] 12/95; vol 13

13. Hepatic Toxicity in Rats Following IV Administration of DAB_{389}IL-2 for 28 Days with a Recovery Period: An Exploratory Study; report #95163; performed at Seragen (non-GLP); lot [redacted] 2/96; vol 14

14. Hepatic Toxicity in Mice Following IV Administration of DAB_{389}IL-2 for 28 Days with a Recovery Period: An Exploratory Study; report #95164; performed at Seragen (non-GLP); lot [redacted] 7/96; vol 15

15. A 6-Week Toxicity Study Using SD Rats Given Intermittent IV Injections of ——— and a Companion Blood Level Study; report #96059; performed at ——— (per GLP); lot ———, 9/96; vol 17

16. A 28-Day Study in SD Rats Comparing the Pathological Changes Caused by DAB,,,IL-2 and Two Analogs of $DAB_{389}IL-2$ with Greatly Reduced Activity ——— and - - report #96131; performed at Seragen (non-GLP); lot- ——— ($DAB_{389}IL-2$), ——— ———; 1/97; vol 21

17. Serial Histopathological Examination of SD Rats Following IV Administration of $DAB_{389}IL-2$; report #96150; performed at Seragen (per GLP); lot ——— 5/97; vol 21

Reports Not Included in the BLA (from IND ———

1. Toxicity of DAB,,,IL-2 Administered IV for 4 Weeks in Mice: An Exploratory Study; report #94014; performed at Seragen (non-GLP); lot #unknown

2. Toxicity of DAB,,,IL-2 Administered IV for 2 Weeks in Mice: An Exploratory Study with a Recovery Period; report #94015;performed at Seragen (non-GLP); lot #unknown

Reports Not Included in the BLA (from IND ———

3. In Vivo Biological Activity of $DAB_{389}IL-2$ Final Drug Product, and ——— Administered IV to Female Rats; report #94122; performed at Seragen (non-GLP); lot ——— (final product); ——— . ——— ; ——— ———

4. Immunogenicity of DAB,,, IL-2 Final Drug Product, ——— , and ——— Administered IV to Female Rats; report #95092; performed at Seragen (non-GLP); lot ——— (final product); ——— . ———

Toxicolocrv Studies

In July 1994, the measurement of protein was changed from the ——— assay to the ——— (chromatographic) assay. For all preclinical studies, the sponsor has recalculated the dose levels based on ——— measurement.

1. Toxicity of DAB_{389}IL-2 Administered IV for 10 Days in Naive and Preimmunized Mice: An Exploratory-Study
Species : ICR ♀ mice
Dose Levels: 0, 1000, 3000 kU/kg/day (44, 131 μg/kg/day - BCA) (36, 106 μg/kg/day - ———
Route/Duration: IV/daily for 10 days
To address the theoretical concern that the presence of pre-existing Abs to diphtheria toxoid could potentially change the toxicity profile of the protein

Methods: Forty mice were preimmunized with diphtheria toxoid (50 μg/mouse), given SC, once weekly for 3 weeks. Two weeks after the last injection, preimmunized and naive mice were IV injected with DAB_{389}IL-2, followed by kill on day 11. Clinical chemistry and histopathology were performed.

Findings:
High levels of antibodies to DAB_{389}IL-2 were found in some mice killed after preimmunization (not exposed to test material). Naive mice dosed with DAB_{389} lost weight during the study. The 131 μg/kg naive mice had elevated LDH, AST, and ALT levels compared to controls. The 131 μg/kg/d naive mice had hepato-cellular necrosis (10/10) and renal tubular necrosis (4/10).

2. Toxicity, Clearance, & Efficacy of DAB_{389}IL-2 (——— Formulation vs. Citrate Formulation) in Rats: A Summary Report
Species: SD rats; C57BL/6 mice
Dose Levels: Toxicity & Clearance Studies - 0. 1000, 3000 kU/kg/day (42, 128 μg/kg/day - ——— 33, 101 μg/kg/day - ——— = citrate
1000, 3000 kU/kg/day (56, 169 μg/kg/day - - 42, 126 μg/kg/day ---------- = ———— :

Efficacy Studv - 0, 500, 2000 kU/kg/day (22, 87 μg/kg/day - ——— 17, 68 μg/kg/day - ——— = citrate
500, 2000 kU/kg/day (28, 113 μg/kg/day - ——— 21, 84 μg/kg/day -

Route/Duration: IV/daily for 10 days
Formulation changes [old = ——— new = citrate] implemented to increase the purity & stability of DAB,,, - resulted in a 20% increase in monomer - with increased in vitro activity

Methods: Toxicity - SD ♀ rats were IV injected with 1000 or 3000 kU/kg/day for 10 days, followed by kill on day 11. Clinical pathology and histopathology (liver, kidney) were performed.

Findings:
Ruffled haircoats; thin; early deaths/kills (100%) - 3000 kU/kg
↓ BWs - all DAB,,, grps
↑ red cell mass - 1000 kU/kg - likely due to dehydration
No hematology obtained on the 3000 kU/kg rats
↓ glucose, albumin - ≥1000 kU/kg
↑ BUN, AST, ALT, GGT, LDH, creatinine, bilirubin - 3000 kU/kg
_____ formulation changes appeared more severe

Mild hepatocellular necrosis [19/20 at 3000 kU/kg]; hepatic regeneration [9/20 at 1000 kU/kg; 19/20 at 3000 kU/kg]; mild/moderate renal tubular epithelial cell necrosis [10/20 at 1000 kU/kg; 19/20 at 3000 kU/kg]; renal tubular regeneration [20/20 at 1000 kU/kg; 16/20 at 3000 kU/kgl.
Incidence higher for _____-formulation

Clearance - SD ♀ rats were singly IV injected with 1000 or 3000 kU/kg, followed by blood collection; and immunoreactive levels (via ELISA) & bioactive levels (via in vitro bioassay) were determined.

Findings:

Table 10
Pharmacokinetic Parameters Associated with Clearance - Bioassay
(Mean ± Standard Deviation)

Parameter	Units	Test Group: Low-dose DAB389IL-2		High-dose DAB389IL-2	
C_{p0}	kU/ml	93 ± 22	170 ± 104	442 ± 316	288 ± 94
Vd	ml/kg	11 ± 3	7 ± 3	11 ± 9	11 ± 4
$t_{1/2}$	min	32 ± 11	24 ± 9	50 ± 17	41 ± 12
AUC	kU/ml x min	3566 ± 1265	3391 ± 806	17721 ± 5784	15874 ± 3108
Clp	ml/kg x min	0.31 ± 0.10	0.32 ± 0.08	0.18 ± 0.05	0.19 ± 0.04

Table 11
Pharmacokinetic h - n Associated with Clearance , ELISA
(Mean ± Standard Deviation)

Parameter	Units	Test Group: Low-dose DAB389IL-2		High-dose DAB389IL-2	
C_{p0}	µg/ml	1.0 ± 0.3	1.1 ± 0.2	5.0 ± 0.5	4.0 ± 0.3‡
Vd	ml/kg	54 ± 11	38 ± 5‡	34 ± 3	32 ± 2
$t_{1/2\alpha}$	min	3 ± 3	2 ± 1	5 ± 2	5 ± 4
$t_{1/2\beta}$	min	41 ± 9	46 ± 11	45 ± 7	46 ± 9
AUC	µg/ml x min	40 ± 10	43 ± 11	130 ± 9	143 ± 23
Clp	ml/kg x min	1.46 ± 0.32	1.03 ± 0.30‡	1.30 ± 0.09	0.91 ± 0.15‡

‡$p < 0.05$; compared to corresponding dose of Lot ' _____

Comment:
● [Per the sponsor] Although the citrate formulation contained ~30% more protein, ELISA-determined sera levels were comparable between the two formulations. Possibly the ELISA detects only bioactive, monomeric material and not the total DAB,,, protein. Correction for monomeric content results in comparable amounts of protein for the two formulations.

Efficacy - C57BL/6 mice were IV injected with 10^6 CP3 cells, followed by IV injection of 500 or 2000 **kU/kg/day** of DAB,,, for 10 days. Mice were observed to day 104 for tumors & survival.
Findings: No differences in survival time were noted between [redacted] & citrate formulations. Mean survival times were -36 (control), 81 (500 **kU/kg**), & 74 (2000 **kU/kg**) days. The 2000 kU/kg mice displayed toxicity due to DAB,,, - weight loss & early death in 3/20 mice.

3. Immunotoxicity of DAR,,,IL-2 Administered IV for 10 Days in Mice: A Summary Report
Species: $B6C3F_1$ mice
Dose Levels: 0 (vehicle), 44 µg/kg/day [redacted] (36, 24 µg/kg/day - Positive control: cyclophosphamide (200 **mg/kg** - single dose on day 10, except for the DTH model - at 75 **mg/kg/day** for 10 days)
Route/Duration: IV/10 days + kills on day 11
The **intent** of **this experiment was to determine whether a non-toxic dose of DAB_{389}IL-2 affected resting cells in the immune system.**

Methods: Ex vivo - mitogen stimulation, MLR, NK cell and macrophage cytotoxicity - and in vivo - SRBC antibody formation, DTH, resistance to B16 melanoma implantation and resistance to Listeria infection - parameters were evaluated.

Findings:
Cyclophosphamide inhibited the immune response in' all tests except DTH [possibly reflective of a regaining of immuno-competence by the time of the secondary immunization], while DAB_{389}IL-2 did not inhibit the immune response in any test.

4. Comparative Toxicity Evaluation of DAB_{486}IL-2 vs. DAB_{389}IL-2 Administered IV to Mice
Species: CD-1 mice (10 ♀s/grp)
Dose Levels: 0, DAB_{486}IL-2 - 0.25, 0.5, 1.0 mg/kg/day ----- & 204, 408, 815 mg/kg/day [redacted]; DAB_{389}IL-2 - 0.025, 0.05, 0.1, 0.213, 0.425, 0.85 mg/kg/day A--- & 19, 38, 77, 164, 327, 653 mg/kg/day -
Route/Duration: IV/14 days + kills on day 15
[Per the sponsor] Only ♀s were used as they have been shown to be more sensitive to DAB_{486}IL-2.

[Per the sponsor] DAB,,, IL-2 is lo-fold more biolcgically active per unit weight than DAB_{486}IL-2.

Methods: Clinical signs, body weights, food consumption, ophthalmology, clinical pathology (terminal), and gross and histopathology were performed.

Findings:
Deaths
DAB_{486}IL-2 - 1 mg/kg - one on day 9 & one on day 12
DAB_{389}IL-2 - 0.425 mg/kg - 10/10 by **day 9; 0.85** mg/kg - 10/10 by day 5

Hypoactivity, abnormal breathing, thinness - seen in animals that died.
↓ BW - 1 mg/kg DAB_{486}IL-2; ≥0.1 mg/kg DAB_{389}IL-2
↓ weight gain - all Rx groups -

↑ BUN, creatinine - ≥0.425 mg/kg DAB,,, IL-2 [↓ glomerular filtration]
↑ AST, ALT - ≥0.5 mg/kg DAB_{486}IL-2 & ≥0.1 DAB_{389}IL-2
↑ AlkP - 0.85 mg/kg DAB_{389}IL-2
↓ lymphocytes - DAB_{486}IL-2

Kidnev - tubular necrosis, dilatation, eosinophilia - Rx groups - dose-related in incidence & severity

Liver - single cell necrosis & hepatocellular necrosis, w/ fatty change - ≥0.5 mg/kg DAB,,, IL-2 & ≥0.1 mg/kg DAB_{389}IL-2
Extramedullary hematopoiesis - Rx groups - dose-related
[Lesions noted at 1.0 mg/kg DAB,,, IL-2 were comparable to those noted at 0.1 mg/kg DAB_{389}IL-2]

Adrenal - cortico-medullary junction necrosis - dose-related - both materials
[Lesions noted at 1.0 mg/kg DAB,,, IL-2 were comparable to those noted at 0.1 mg/kg DAB_{389}IL-2]

Lung - hemorrhage/acute inflammation - all Rx groups, with necrosis & chronic inflammation - 1.0 mg/kg DAB_{486}IL-2 and ≥0.1 mg/kg DAB_{389}IL-2

Injection Site - perivascular hemorrhage/inflammation - high doses of both materials

Lymph Nodes - lymphoid hyperplasia and/or depletion - Rx groups - dose-related

Spleen/Thymus - lymphoid depletion - 1.0 mg/kg DAB_{486}IL-2 and ≥0.1 mg/kg DAB_{389}IL-2 - dose-related

Bone Marrow - hematopoiesis - Rx groups - dose-related

5. A Study to **Compare** the Clearance Pattern **and Toxicity of Two Related Test Articles Following Administration by IV Injection to Cynomolgus Monkeys**
Species: cyno monks (3/sex/grp)
Dose **Levels:** $DAB_{486}IL-2$ -250 µg/kg/day ——; 204 µg/kg/day ——
$DAB_{389}IL-2$ - 40 µg/kg/day ——, 31 µg/kg/day ——
Route/Duration: IV/14 days + 14-day nonRx observation period

Methods: Clinical signs, body weights, ophthalmology, clinical pathology (baseline and days 8, 15, 29), antibody titers, and PK analysis were performed.

Findings: [note that no control grp was run]
Death one $DAB_{389}IL-2$ ♀ - found comatose & killed on day 29
↓ WBC, lymphocytes, red cell mass, platelet;
↑ monocytes - day 15
↓ WBC, lymphocytes - day 29
↑ AST, ALT, creatinine - days 8, 15, and/or 29
↓ albumin - day 15
↓ glucose - day 29

Histo - lymphoid depletion in LNs; acute inflammation in liver & gallbladder; extramedullary hematopoiesis in liver; inflammation of colon & rectal mucosa; biliary hypertrophy; ↑ erythroid cells and ↑ immature megakaryocytes in BM
[severe intra-abdominal infection/anemia]

Survivors
↓ BW - both grps, with some resolution by day 29
Injection site bruising - both grps - healed by day 17
Pale mucus membranes, dehydration - both grps

↓ WBC, lymphocytes; ↑ monocytes - day 15 - both grps
↑ AST, ALT, creatinine - days 8, 15 - both grps
↓ albumin - day 15
↓ glucose - day 15
[Recovery trend by day 29 for all parameters]

Clearance rate of $DAB_{486}IL-2$ was 4-fold faster than $DAB_{389}IL-2$. Both Rx grps developed antibody titers to diphtheria toxin and the respective fusion protein itself. Only animals exposed to $DAB_{486}IL-2$ developed significant neutralizing antibody levels and significant anti-IL-2 ELISA titers.

6. Effect of Diet on the Toxicity of $DAB_{389}IL-2$ Administered IV to Rabbits
Species: NZW ♂ rabbits (5/grp)
Dose Levels: Standard diet = 1000 kU/kg $DAB_{389}IL-2$
High cholesterol diet = 0, 1000 kU/kg $DAB_{389}IL-2$; 41 µg/kg/day ——, 31 µg/kg/day ——
Route/Duration: IV/7 days + 14-day nonRx observation period + kill

[Per the sponsor] In earlier efficacy studies in rabbits that were on high cholesterol diets, doses of 1000 kU/kg/day resulted in mortality, whereas the same dose given to rodents was nontoxic. This study was an attempt to determine the effect of diet on $DAB_{389}IL-2$ toxicity.

Methods: Clinical signs, body weights, food consumption, clinical pathology (baseline and days 8, 15, 22) and gross and histopathology were performed.

Findings:
Deaths -
Standard diet (DAB) -one died on day 8/two killed on days 15 & 16
High chol. diet (DAB) - one killed on day 8/two killed on day 15

Animals were dehydrated, thin, weak, had shallow respiration, with pale mucous membranes. One standard diet rabbit also showed trembling, abnormal gait, and hindlimb splay prior to death.

↑ BUN, creatinine (10-fold baseline), AST, ALT, glucose, phosphorus, triglycerides

Survivors
Clinical signs were noted in both DAB grps - hypoactivity, thinness - noted beginning week 1

↓ BW (2-16%) during week 1 - all grps; continuing to ↓ for the DAB grps for the study

↓ food consumption - all grps from week 1 to week 2; large ↑ for week 3 - most notable in the DAB grps

↓ lymphocytes; ↑ basophils, PLTs - days 8/15 - DAB grps
↓ WBCs, red cell mass - day 22 - standard diet (DAB)
↑ basophils - day 22 - high chol. diet (DAB)
↓ red cell mass - days 15, 22 - high chol. diet (DAB)

↑ cholesterol - high chol. diet grps
↑ BUN, creatinine, AST, ALT, phosphate, glucose; ↓ protein, calcium - days 8, 15 - DAB grps

All clin path parameters trended toward baseline by day 22.

Histo
Kidney - tubule dilatation, focal mineralization, tubule degeneration, tubule eosinophilia, tubule cell necrosis, cytoplasmic lipid vacuoles, tubule lumen droplets (hyaline), basophilic epithelial cells in tubule (indicative of cell division)

Interstitial inflammation - control & DAB grps - due to Encephalitozoon infection

Liver - hepatocellular fatty change, extramedullary hematopoiesis, hepatocellular necrosis, centrolobular hepatocellular hydropic degeneration, and bile pigment.

Biliary proliferation and periportal inflammatory cells - control & DAB grps - due to Encephalitozoon infection

Lung - interstitial inflammation, and acute/chronic inflammation Lipid histiocytosis - control & high chol. DAB grp - reflective of the high chol. diet

Adrenal - necrosis, inflammation, hemorrhage, leukocyte infiltrates, and mineralization

Cortical hyperplasia and cytoplasmic lipid vacuoles (cortex cells) - control & high chol. DAB grp

With the exception of the anemia that was exacerbated by the high chol. diet, the hepatorenal, systemic, and associated toxicities of the fusion protein were similar in prevalence and severity in the different diet groups.

7. A Comparison of DAB_{389}IL-2 Toxicity and PK in Male and Female Rats Following IV Bolus Administration: A Summary Report
Species: SD rats
Dose Levels: 0, 30, 90 µg/kg/day ——— , 31, 94 µg/kg/day ——— and 75 µg/kg/day ———
Route/Duration: IV/10 days
PK data were assessed at 25 & 75 µg/kg

Methods: Clinical signs, body weights, clinical pathology (at kill) and gross and histopathology (liver & kidneys) were performed.
PK - Bioassay (measures the ability of DAB_{389}IL-2 to inhibit protein synthesis in a human IL-2R expressing cell line, with a lower limit of detection of 20 U/mL [I ng/mL])

ELISA (with a lower limit of detection of 40 ng/mL)

Findings: 30, 75, 90 μg/kg grps:
Rough haircoats, hypoactive - ≥75 μg/kg
↓ **BWs** - DAB grps - the ♀s lost more weight than the ♂s

Sera levels of bioactive/immunoactive **DAB_{389}IL-2** were comparable between the sexes

↑ AST, ALT - DAB grps

Kidney - mild to severe necrosis of tubular epithelial cells; tubular regeneration - DAB grps

Liver - mild to moderate infiltration of inflammatory cells; scattered necrotic hepatocytes - ≥75 μg/kg

PK -

Table 3.8
Pharmacokinetic Parameters - Bioassay
(Mean ± Standard Deviation)

DAB_{389}IL-2 - 25 μg/kg (Preclinical Study No. 2109-04,7-94)

Parameter	Units	Male	Female
$t_{1/2}$	min	34 ± 16	20 ± 5
Vd	mL/kg	11 ± 3	11 ± 5
Cmax	U/mL	64410 ± 14186	78052 ± 47615
AUC	U/mL x min	2940675 ± 3584%	2313144 ± 1787031
Clp	mL/kg x min	0.24 ± 0.03	[illegible]

DAB_{389}IL-2 - 75 μg/kg (Preclinical Study No. 2109-04,7-94)

Parameter	Units	Male	Female
$t_{1/2}$	min	28 ± 7	20 ± 7
Vd	mL/kg	8 ± 2	11 ± 5
Cmax	U/mL	27652, ± 83073	220038 ± 71916
AUC	U/mL x min	1530458 ± 2497729	8473450 ± 3181537
Clp	mL/kg x min	0.19 ± 0.04	0.29 ± 0.16

DAB_{389}IL-2 - 25 μg/kg (Preclinical Study No. 2109-04,7-94)

Parameter	Units	Female - anesthetized	Female - conscious
$t_{1/2}$	min	20 ± 5	17 ± 8
Vd	mL/kg	11 ± 5	12 ± 8
Cmax	U/mL	78052 ± 47615	71239 ± 31614
AUC	U/mL x min	2313144 ± 1787031	1567672 ± 361 178
Clp	mL/kg x min	0.39 ± 0.14	0.46 ± 0.12

Table 3.9
Pharmacokinetic Parameters - Product RLISA
(Mean ± Standard Deviation)

DAB_{389}IL-2 - 25 μg/kg (Preclinical Study No. 2109-04,7-94)

Parameter	Units	Male	Female
$t_{1/2}$	min	36 ± 5	26 ± 6
Vd	mL/kg	43 ± 7	51 ± 14
Cmax	ng/mL	591 ± 96	516 ± 182
AUC	ng/mL x min	32936 ± 5524	19405 ± 13424
Clp	mL/kg x min	0.78 ± 0.15	1.64 ± 0.6,

DAB_{389}IL-2 - 75 μg/kg (Preclinical Study No. 2109-04,7-94)

Parameter	Units	Male	Female
$t_{1/2}$		31 ± 8	21 ± 8
Vd	min	31 ± 4	36 ± 10
Cmax	mL/kg	2450 ± 283	2221 ± 663
AUC	ng/mL x min	119124 ± 26576	84322 ± 26470
Clp	mL/kg x min	0.65 ± 0.14	0.96 ± 0.26

DAB_{389}IL-2 - 25 μg/kg (Preclinical Study No. 2109-04,7-94)

Parameter	Units	Female - anesthetized	Female - conscious
$t_{1/2}$	min	26 ± 6	13 ± 3
Vd	mL/kg	51 ± 14	45 ± 8
Cmax	ng/mL	526 ± 182	564 ± 113
AUC	ng/mL x min	19405 ± 13424	12814 ± 4632
Clp	mL/kg x min	1.64 ± 0.68	2.19 ± 0.55

Conclusion: No notable gender specificities

8. Acute Toxicity of Citrate Formulated DAB_{389}IL-2 Following IP Injection in Mice and Guinea Pigs: Summary of Step-Down Studies
Species: ♀ ICR mice (<25 g); ♀ Hartley guinea pigs (<400 g)
Dose Levels: 0, 7.5, 15, 30 μg/mouse of each lot
0, 75, 150, 450 μg/guinea pig of each lot
Route/Duration: IP/single injection + kill on day 8

This was a citrate formulation, while earlier studies were done with a ———— formulation. The apparent intent of this study was to select doses (dilutions) for the General Safety Test.

Findings:
Mice - Rough haircoats, deaths - 215 μg/mouse
↓ BWs - DAB grps

Guinea Piss - Deaths - 450 μg/pig
↓ BWs - DAB grps

9. A Comparison of DAB,,,IL-2 Toxicity and PK in Normal Mice and Mice with IL-2 Receptor Expressing Tumors: A Summary Report
Species: ♀ C57BL/6 mice
Dose Levels: 0, 20, 60 μg/kg/day
Route/Duration: IV/single injection for PK study & injection on days 11-20 in tumor study

Methods: On day 0, tumor mice were IV injected with 10^6 CP3 cells [murine IL-2R expressing lymphoma], followed by injections of $DAB_{389}IL-2$ on days 11-20, followed by kill on day 21.

Findings:

20 μg/kg
↓ BWs - normal & tumor bearing mice
↓ AST, ALT, LDH, total bilirubin - tumor bearing mice

Necrosis (mild) of renal tubular epithelial cells - normal (7/10 mice) & tumor (3/10 mice)

60 μg/kg
↓ BWs - normal & tumor bearing mice

Rough haircoats; ↑ AST, ALT, total bilirubin, LDH; ↓ glucose - normal & tumor bearing - less severe for the tumor bearing mice

Necrotic hepatocytes - normal (7/10 mice) & tumor (3/10 mice)
Necrosis of renal tubular epithelial cells - normal (10/10 mice) & tumor (7/10 mice)
Hepatitis - 2/10 tumor mice

PK - analyzed by bioassay [lower limit of detection = 20 U/mL (1 ng/mL)] and by ELISA [lower limit of detection = 40 ng/mL] -

Sera levels were not significantly different between normal & tumor bearing mice, however mean levels were slightly higher for normal mice:

Table 341

Serum Concentrations - Bioassay (U/mL)
(Mean ± Standard Deviation)

$DAB_{389}IL-2$ - 20 μg/kg/d

Time (min)	Tumor Cells (-)	Tumor Cells (+) Day 11‡	Tumor Cells (+) Day 20
5	25693 ± 8829	22710 ± 3322	35019 ± 10409*
15	24746 ± 14105	14446 ± 4437*	17892 ± 3628
30	8334 ± 3749	5252 ± 1916*	6201 ± 2214

*p <0.05 compared to group without tumors (ANOVA of individual time points)
‡p <0.05 compared to group without tumors (repeated measures ANOVA for time course)

Table 342

Serum Concentrations - Product ELISA (ng/mL)
(Mean ± Standard Deviation)

$DAB_{389}IL-2$ - 20 µg/kg/d

Time (min)	Tumor Cells (-)	Tumor Cells (+) Day 11‡	Tumor Cells (+) Day 20‡
5	305 ± 41	191 ± 28'	236 ± 57*
15	155 ± 45	127 ± 26	122 ± 24*
30	65 ± 23	77 ± 20	70 ± 32

*p <0.05 compared to group without tumors group (ANOVA of individual time points)
‡p <0.05 compared to group without tumors (repeated measures ANOVA for time course)

10. A Comparison of the Toxicity and PK of $DAB_{389}IL-2$, _____ and _____ in Rats: A Summary Report

_____ - has a single amino acid change which reduces the enzymatic activity of the DT portion of the molecule

_____ - binds poorly to IL-2R due to a deletion in the IL-2 part of the molecule

Thus these constructs have a reduced ability to inhibit in vitro protein synthesis in IL-2R expressing cell lines.

Species: SD ♀ rats
Dose Levels: $DAB_{389}IL-2$ - 0, 50 µg/kg/day (1395 kU/kg/day)
_____ - 200 µg/kg/day (1 kU/kg/day)
_____ 200 µg/kg/day (10 kU/kg/day)
Route/Duration: IV/10 days + kill on day **11**

Methods: PK (postdose #1), clinical chemistry and histopathology (liver, kidney)

Findings:
[Compared to vehicle control]:
$DAB_{389}IL-2$ rats - rough haircoats; ↓ weight; ↑ AST, ALT, LDH; necrotic hepatocytes (6/10), necrotic renal tubular cells (10/10), and renal tubular regeneration (6/10)
PK (via bioassay & ELISA)

_____ rats - exhibited similar toxicity findings, but of less severity & frequency compared to $DAB_{389}IL-2$
Necrotic hepatocytes (3/10), necrotic renal tubular cells (9/10), and renal tubular regeneration (3/10)

PK (via bioassay, as the product is missing the IL-2 epitope to be recognized in the ELISA)

_____ rats - no toxicity
PK- - below the level of detection in the bioassay; ELISA used

_____ _____ - cleared -3-fold faster from the circulation than $DAB_{389}IL-2$

Conclusion: Some of the toxic effects noted can be attributed to non-specific activity related to the toxin portion of the fusion protein, as although ——— binds poorly to IL-2R, the enzymatic activity of the DT portion of the molecule was not altered

11. Toxicity of DAB_{389}, ——— and ——— Administered IV for 10 Days in Mice: A Comparison Study---
Species: ICR ♀ mice
Dose Levels: $DAB_{389}IL\text{-}2$ - 0, 2500 U/kg/day (108 µg/kg/day)
——— - 136 µg/kg/day
——— - 138 µg/kg/day
Route/Duration: IV/10 days + kill on day 11

Methods: Clinical chemistry and histopathology done at kill.

Findings:
Compared to vehicle control:
$DAB_{389}IL\text{-}2$ mice - ↓ weight; ↑ AST, ALT, LDH; scattered necrotic hepatocytes (10/10) & necrotic renal tubular cells (1/10)

12. Evaluation of Toxicity and PK of $DAB_{389}IL\text{-}2$ in Cynomolgus Monkeys Following 30 Days of IV Administration
Species: cyno monkeys (2-3/sex/grp), with nondetectable/low anti-$DAB_{389}IL\text{-}2$ Abs & anti-IL-2 Abs
Dose Levels: 0, 2.5, 10, 25 µg/kg/day
Route/Duration: IV/variable regimens - see table:

Group	Number Males/ Females	Dose Level of $DAB_{389}IL\text{-}2$ (µg/kg/day)	Conc. (µg/ml)	Dose Volume ml/kg	Days of Dosing	Day of Necropsy
1A	1/1	0	0	0.167	1 to 30	31
1B	2/2	0	0	0.167	1 to 44	45
2	3/3	2.5**	15	0.167	1 to 30	31
3	3/3	10.0	60	0.167	1 to 30	31
4A	3/3	25.0	150	0.167	1 to 29	29
4B	2/2	25.0	150	0.167	1 to 15	16
4C	2/4*	25.0	150	0.167	1 to 15 & 30 to 44	45

* An additional two animals were added because of deaths in Group 4C on Days 11 and 19
** Because of a dose calculation error, Group 2 animals received 2.5 µg/kg/day of test material rather than 1 µg/kg/day as required by protocol.

Note that there was a 2-wk cessation of dosing after 15 days at 25 µg/kg, followed by another 2-wk dosing period (grp 4C)

Methods: Clinical signs, BWs, ECGs, ophthalmology, clinical pathology (baseline & days 7, 14, 21, 28, and at kill), anti-$DAB_{389}IL\text{-}2$ & anti-IL-2 Abs, PK, and gross & histopathology were performed,

Findings:
A total of 7 unscheduled deaths occurred:
2.5 µg/kg - 1/3 ♂s - day 18
10 µg/kg - 1/3 ♂s - day 15
25 µg/kg - 1/7 ♂s - day 15 (grp 4B)
25 µg/kg - 4/10 ♀s - days 11 (grp 4C), 19 (grp 4C), 27 (grp 4A), 42 (grp 4C)

The 2.5 µg/kg ♂ had a pre-existing splenic mass, most likely an abscess, resulting in septicemia.-

The 10 µg/kg ♂ died due to a collar "caught" in the cage during PK sampling (no further explanation given). ↑ retics, AST, ALT, trigly, LDH; ↓ red cell mass, lymphocytes; pigmentation w/ minimal hypertrophy of Kupffer cells

One 25 µg/kg ♂ - emaciated, dehydrated; thymic lymphoid atrophy, endothelial cell hyperplasia of splenic red pulp, germinal center depletion of lymphoid follicles of spleen & LNs

One 25 µg/kg ♀ - ↓ lymphocytes, red cell mass, total protein, albumin; ↑ AST, ALT, LDH; disseminated CMV infection (endothelial cell & macrophage cytomegaly in many organs, w/ intranuclear & intracytoplasmic inclusions)

One 25 µg/kg ♀ - fecal cultures positive for *Shigella* & *Campylobacter;* thymic lymphoid atrophy; colonic mucosa thickening, gas & fluid in colon & cecum; renal tubular degeneration & glomerular hypercellularity, endothelial cell hyperplasia of splenic red pulp (w/ germinal center depletion), single-cell degeneration of liver, lymphadenitis of LNs

One 25 µg/kg ♀ - emaciation; thymic lymphoid atrophy; cytoplasmic vacuolization & leukocytic foci of liver, splenic germinal center depletion, eosinophilic hyperplasia of BM, mononuclear infiltration of LNs & spleen

One 25 µg/kg ♀ - diarrhea, emaciation; thymic lymphoid atrophy; cytoplasmic vacuolization of liver, splenic & LN germinal center depletion, myeloid hyperplasia of BM, mononuclear infiltration of many organs (including choroid plexus, iris, adrenal)

Clinical signs - ↓ BWs, diarrhea, dehydration, anorexia, hypoactivity - noted in weeks 2/3; dose- & regimen-related [2/6 monks (10 µg/kg), 6/6 (grp 4A), 2/4 (grp 4B), 4/6 (grp 4C)]

Nutritional supplements, Pepto Bismol required to counter weight loss/dehydration/diarrhea - 1/6 monks (2.5 µg/kg); 5/6 (grp 4A), 1/4 (grp 4B), 4/6 (grp 4C) - total of 6/11 monks treated were unscheduled deaths

ECGs, ophthalmology - no abnormalities

↓ lymphocytes (dose-related) [returned to baseline in grp 4C by day 21/28]
↓ red cell mass; ↑ retics - 25 μg/kg

↑ AST, ALT, globulin, LDH; ↓ albumin, total protein, GGT, calcium - ≥10 μg/kg

Histo - ≥10 μg/kg
Mononuclear cell infiltrate - spleen, LNs, liver, kidney, brain, adrenal, heart, lung, muscle, &-brain (choroid plexus)
Lymphoid depletion - spleen, thymus, LNs
Liver - cytoplasmic vacuolization, necrosis, single-cell degeneration, Kupffer cell pigmentation & hypertrophy
Vasculitis [one grp 2 & one grp 4A animal]
Kidney - glomerular hypercellularity
Spleen - endothelial cell hypertrophy (red pulp), germinal center depletion, lymphoid atrophy
Lung - pleural fibrosis, edema, pneumonia, congestion
Brain - meningeal hemorrhage [two grp 4C animals]
LNs - lymphoid atrophy, germinal center depletion
Testicular degeneration/BM hyperplasia (myeloid, eosinophilic) - secondary effects

Note: the most severe alterations occurred in Grp 4A. Most of the Rx monks displayed Abs (some neutralizing) to the fusion protein. Ab response to the IL-2 epitope was low.

A NOEL was not achieved.

13. Hepatic Toxicity in Rats Following IV Administration of $DAB_{389}IL\text{-}2$ for 28 Days with a Recovery Period: An Exploratory Study
Species: Hsd:SD ♀ rats
Dose Levels: 0, 10, 25 μg/kg/day
Route/Duration: IV/5x/week for 2 wks + 4-wk recovery or 5x/week for 4 wks + 8-wk recovery

Methods: Clinical signs, BWs, clinical chemistry (terminal), anti-$DAB_{389}IL\text{-}2$ Abs, PK, and gross & histopathology (liver, spleen, kidneys, lungs, brain) and P450 analysis (liver) were performed

Findings:
Deaths - 1/35 at 10 μg/kg x 4 wks (day 24); 4/35 at 25 μg/kg x 4 wks (day 29)

Kills - 2/35 at 10 µg/kg x 4 wks (day 23/24); 11/35 at 25 µg/kg x 4 wks (day 22/23/25/26/33/37) - noted to be thin & weak; ↑ BUN (1-1.5-fold); chronic inflammation in lungs, lymphoid hyperplasia (spleen), mononuclear cell infiltrates (liver), basophilic & dilated tubules (kidney)

Clinical signs - rough coats, thin, weak, hypoactive - 10 µg/kg (2 wks) & 25 µg/kg (2, 4 wks) - recovery

↓ BW gain - 10 µg/kg (2 wks) & 25 µg/kg (2, 4 wks) - recovery

↓ phosphorus, sodium; ↑ GGT - 10 µg/kg (2 wks) - **day** 43
↓ phosphorus, sodium; ↑ GGT - 25 µg/kg (2 wks) - day 43

↓ phosphorus, A/G ratio; ↑ ALT, LDH - 10 µg/kg (4 wks) - days 29, 59, a5
↓ A/G ratio; ↑ AST, ALT, BUN - 25 µg/kg (4 wks) - day 29
↓ Cl, sodium; ↑ GGT, LDH - 25 µg/kg (4 wks) - days 59, 85

↓ Cytochrome P450 levels - 25 µg/kg x 4 wks

Abs - low titers were sporadically seen in the 10 µg/kg rats - ↑ with the 4-wk grp
Low titers noted w/ 25 µg/kg x 2 wks
↑ titers & neutralizing response - 25 µg/kg x 4 wks - days 30, 59, a5

Histo -
Lung - chronic inflammation (macrophages & WBCs) - **all** DAB grps - days 15, 29 - ↑ incidence in 10 & 25 µg/kg (4 wks) and ↑ severity in 25 µg/kg (4 wks) [>50% involvement of pulmonary parenchyma & emphysema for some] - not bacterial in origin
Perivascular edema, vascular congestion, hyperplasia of bronchiolar epithelium - no recovery in 25 µg/kg (2 & 4 wks)

Spleen - lymphoid hyperplasia - 25 µg/kg (4 wks) - trend toward resolution
Liver - ↑ multifocal mononuclear infiltrates (no fibrosis) - 25 µg/kg (4 wks) - trend toward recovery
Kidney - ↑ basophilic tubules (degeneration) - 25 µg/kg (4 wks) - no recovery - **nephroblastoma noted in 1 (10 µg/kg x 2 wks - day 43) & 1 (25 µg/kg x 4 wks - day 85) rat (2.6% incidence)**
[Per the sponsor] Such neoplasms occur ~0.2% in SD rats (based on literature & historical data) and should be considered malignant. **This is the first reported finding of this malignancy with this material.**

Vascular thrombi - Noted in one 10 µg/kg ♀ - lungs (2 wks)
Noted in one 10 µg/kg ♀ (dead on day 29) - brain, lungs, liver, kidneys, vessels

Noted in one 25 µg/kg ♀ (2 wks) - lungs

Noted at 25 µg/kg - lungs (one ♀, 2 wks & one ♀, dead on day 26); brain (one ♀, dead on day 22)

Brain hemorrhage - Noted in two 25 µg/kg ♀s (dead on day 22/25)

A NOEL was not achieved.

Comment:
● Noted in the P/T review of 4/1/96 for this study - based on a cursory review of earlier preclinical studies performed, it appeared that the no effect dose was becoming lower, thus possibly a change in potency (kU) of the material has occurred. At that time, the product reviewer stated that the product now contained [redacted] vs. [redacted] in the previous product. Based on a study done in rats [report #95092; study #3, listed under "other studies"] the -- appears to have deleterious effects similar to those in this study.

14. Hepatic Toxicity in Mice Following IV Administration of $DAB_{389}IL-2$ for 28 Days with a Recovery Period: An Exploratory Study
Species: ICR ♀ mice
Dose Levels: 0, **10**, 25 µg/kg/day
Route/Duration: IV/5x/week for 2 wks + 4-wk recovery or 5x/week for 4 wks + 8-wk recovery

Methods: Clinical signs, BWs, clinical chemistry (terminal), anti-$DAB_{389}IL-2$ Abs, PK, and gross & histopathology (liver, spleen, kidneys, lungs, brain) were performed

Findings:
Deaths - 1/45 at 25 µg/kg x 4 wks (day 17)

Clinical sisns - rough coats, thin - **10** µg/kg (4 wks) & 25 µg/kg (2, 4 wks) - recovery

↓ BW gain - **25** µg/kg (2, 4 wks) - recovery

↑ potassium, **ALT, AST,** total bili (days 29); ↑ GGT (day 43) - 10 µg/kg (2 wks)
↑ GGT (day 59) - 10 µg/kg (4 wks)

↑ AST, ALT, total bili; ↓ globulin (day 15); ↑ GGT (day 43) - 25 µg/kg (2 wks)

↑ potassium, AST, ALT, total bili, globulin (day 29) - 25 µg/kg (4 wks)

Abs - 10 µg/kg (2 wks) - 20% had moderate/high Ab response by day 15, increasing to 60% by day 43 [60% neutralizing]
10 µg/kg (4 wks) - 60% had moderate/high Ah response by day 30 [40% neutralizing]

25 µg/kg (2 wks) - 100% had Ab response [60% neutralizing1 by day 15
25 kg/kg (4 wks) - 100% had Ab response [80% neutralizing] by day 30

Histo -
Spleen - minimal/mild hyperplasia of white pulp - 10 & 25 µg/kg (4 wks) - trend toward resolution
Liver - diffuse hepatocytic vacuolation [glycogen accumulation] - DAB mice - dose-related in severity - noted in recovery at 10 µg/kg (2 wks) & 25 µg/kg (2, 4, wks)

Lung - multifocal granulomatous inflammation - recovery mice - 10 & 25 µg/kg (4 wks)

A NOEL was not achieved.

15. A 6-Week Toxicity Study Using SD Rats Given Intermittent IV Injection6 of ——— and a Companion Blood Level Study
Species: SD rats (15/sex/grp for tox study & 37/sex/grp for TK study)
Dose Levels: 0, 2, 10, 40 µg/kg/day
Route/Duration: IV/5-day dosing + 9-day no-dose phase = 1 cycle; total of 3 cycles were completed
Total of 10/sex/grp were killed at the end of dosing of cycle #3 & 5/sex/grp were killed at the end of the no-dose phase of cycle #3

Methods: Clinical signs, BWs, food consumption, clinical pathology (days 5/6, 19/20 33/34, 42/43), TK profile, Ab development, hepatic cytochrome P450 (day 33), male reproductive endpoints [sperm motion, sperm breakage, sperm concentration], and gross & histopathology were performed.

Findings:
Thickening/reddening of ears - ≥10 µg/kg - beginning -day 28
Hypoactivity, chromorhinorrhea, chromodacryorrhea - sporadically in DAB animals

↓ BWs, food consumption - 10 μg/kg ♀s; 40 μg/kg
No adverse effects on sperm parameters or on hepatic cytochrome P450 levels

↓ WBCs, lymphocytes; ↑ neutrophils - ≥10 μg/kg - days 5/6, 19/20
4 WBCs, lymphocytes - 40 μg/kg ♂s - days 33/34, 42/43

4 ALT (~24-76%), AST (~19-46%), GGT - ≥10 μg/kg - days 5/6, 19/20, 33/34

Abs - both the titer and the incidence (number of rats) increased with dose and cycle of dosing. More ♂ rats had titers of ≥5 than ♀s at ≥10 μg/kg

TK -

Dose (μg/kg)	$AUC_{0-\infty}$ (ng.hr/mL)	C_{max} (ng/mL)	$T_{\frac{1}{2}}$ (hr)	Cl L(hr.kg)
First Dose				
2(♂)	27.90	29.89	1.7	0.072
10	113.6	103.2	2.1	0.088
40	448.2	676.8	3.3	0.089
2(♀)	19.46	29.87	2.5	0.103
10	95.83	104.3	1.7	0.104
40	483.4	519.8	1.2	0.083
Last Dose				
2(♂)	19.22	37.28	1.2	0.104
10	----	20.99	---	-----
40	49.14	233.3	0.5	0.814
2(♀)	12.28	20.47	1.3	0.163
10	47.11	129.2	2.8	0.212
40	----	645.6	---	-----

---- = unable to calculate

The sera levels of $DAB_{389}IL-2$, as well as the AUC, were decreased with the last dose compared to the first dose - which correlated with Ab titers. Males showed a higher reduction in sera levels and AUC, as well as higher Ab titers relative to females.

Dav 34
↑ kidney, spleen weights - 40 μg/kg
4 liver; ↓ adrenal - ≥10 μg/kg

Dav 43
4 spleen; ↓ adrenal weights - ≥10 μg/kg ♂s

Dav 34
Perivascular leukocytic infiltration - kidneys, liver, spleen, lung, prostate, epididymis + other tissues - slight/moderate in 2 µg/kg (15%), 10 µg/kg (50%); slight/severe in 40 µg/kg (90%) - trend toward recovery

↑ adrenocortical vacuolation - DAB grps, with 80% of 40 µg/kg affected - trend toward recovery

Injection site reaction - slightly higher in severity & incidence in DAB grps - recovery

A NOEL was not achieved.

Note that the dosing regimen resulted in a less toxic profile compared to studies in which DAB,, IL-2 was injected daily for 10-30 days - likely due to the development of Ab titers, resulting in accelerated clearance of the molecule.

16. A 28-Day Study in SD Rats Comparing the Pathological Changes Caused by DAB,, IL-2 and Two Analogs of DAB_{389}IL-2 with Greatly Reduced Activity (_____ and _____
Species: Hsd:SD ♀ rats (10/grp)
Dose Levels: 0, 25 µg/kg/dose [DAB_{389}IL-2]; 170 µg/kg/dose [_____], 112 µg/kg/dose [_____]
Route/Duration: IV/5x/week for 4 wks [days 1-5, 8-12, 15-19, 22-26] + kill on day 29

Methods: Clinical signs, BWs, clinical pathology (terminal), and gross & histopathology (liver, kidneys, heart, lungs, spleen, and gross lesions) were performed

Findings:
Deaths - 1/10 - DAB_{389}IL-2 - day 23 [ruffled fur; thin; weak; ↓ BW; chronic active inflammation of lungs]; no abnormal hematology

Clinical signs -
DAB_{389}IL-2 - rough coats, swollen tails, red ears - day 10 thru terminal
↓ BW gain
↑ AST, ALT, potassium, phosphorus

Kidneys - basophilic tubules (associated with early degenerative changes) - 4/10
Liver - mononuclear cell infiltrates - 7/10
Spleen - lymphoid hyperplasia - 2/10
Lungs - inflammation - 6/10 - most severe in this grp

Lungs - inflammation - 1/10

Lungs - inflammation - 4/10

Control
Lungs - inflammation - 5/10

Conclusion: The enzymatic and IL-2 binding domains of the DAB protein need to be intact in order to induce the complete and severe toxicity profile that has been noted in earlier studies.

17. Serial Histopathological Examination of SD Rats Following IV Administration of $DAB_{389}IL-2$
Species: Hsd:SD ♀ rats (24/grp)
Dose Levels: 0, 25, 100 μg/kg/day in naive & preimmune rats
Route/Duration: IV/days 1-14 (25 μg/kg); days 1-3 (100 μg/kg) + kills on days 2, 4, 8, **15, 21, 29, 36, 43**

Preimmune rats - with $DAB_{389}IL-2$ [50 μg/rat/day], SC injection, for 4 days, at -3 weeks prior to IV dosing

Methods: Clinical signs, BWs, Ab titers, clinical pathology (baseline & terminal), cytokines [IL-1β, TNFα, IFNΓ], and histopathology [liver, kidneys, lungs, spleen] were performed.

Findings:
Ruffled fur, hunched, thin - 25 μg/kg (days 8-19), 100 μg/kg (days 6-14) - dose-related; ↑ severity in naive rats

Dyspnea, cyanosis, ataxia - preimmune DAB rats - day 1 - onset & duration was dose-related [recovery by 45 minsl - symptoms noted until day 5

↓ BWs - naive DAB rats

Naive rats - development of low/insignificant titers of anti-$DAB_{389}IL-2$ Abs; no anti-IL-2 Abs
Preimmune rats - no anti-IL-2 Abs & moderate anti-$DAB_{389}IL-2$ Abs at baseline - with dosing, the anti-DAB Ab titers increased and low anti-IL-2 titers appeared

↑ AST - days 2, 4 - 100 μg/kg rats; day 22 for 25 μg/kg rats
No effect on cytokine levels noted

Hepatic multifocal mononuclear cell infiltrates -
25 μg/kg - naive rats - days 15-43
1/24 (100 μg/kg) preimmune rats - days 4, 22

Basophilic/dilated renal tubules; mononuclear cell infiltrates - All DAB grps - starting on day 4 - more severe in naive rats
Chronic/active inflammation in lungs - all grps
Splenic lymphoid hyperplasia - all grps

Other Studies

1. Toxicity of DAB,,,IL-2 Administered IV for 4 Weeks in Mice: An Exploratory Study
Species: ICR ♀ mice
Dose Levels: 0, 250, 1000 U/kg/day (11, 44 kg/kg/day)
Route/Duration: IV/daily for 28 days

Methods: Daily dosing with kill on day 29 [clinical chemistry and histopathology were done].

Findings: 44 μg/kg/d - 1/10 mice had ↑ AST, ALT (2-5-fold higher than controls)

2. Toxicity of DAB,,,IL-2 Administered IV for 2 Weeks in Mice: An Exploratory Study with a Recovery Period
Species: ICR ♀ mice
Dose Levels: 0, 2500 U/kg/day (108 μg/kg/day)
Route/Duration: IV/daily for 14 days + kills on days 15 & 30

Methods: Clinical chemistry and histopathology done at kill.

Findings: [Compared to controls]: **Day 15 -** ↓ weight; ↑ AST, ALT; scattered necrotic hepatocytes (7/10) & necrotic renal tubular cells (1/10)
Day 30 mice - reversible, with exception of 1/6 with ↑ AST, ALT

3. In Vivo Biological Activity of DAB,,,IL-2 Final Drug Product, [redacted] and [redacted] Administered IV to Female Rats
[The final drug contains [redacted] & several [redacted]

Species: SD ♀ rats
Dose Levels: 0, 75 μg/kg/day [2100 kU/kg (final drug); 2700 kU/kg [redacted], 175 kU/kg ([redacted]; 125 kU/kg ([redacted] 110 kU/kg ([redacted] - see table
Route/Duration: IV/daily for 10 days + kill on day 11

Methods: Clinical signs, BWs, clinical chemistry, histopath (kidneys and liver only) were performed

Test Material	Lot	Protein Concentration (µg/mL)	Specific Bioactivity (kU/mg)	(%)
final drug product		138	27786	59
		200	36158	99
		125	2302	2
		168	1495	7
		94	1694	4

*determined by native size exclusion

Findings:
Final drug or ———
Ruffled haircoats - both grps - starting day 7
Thin appearance - ——— grp - starting day 8
One death - ——— - day 10
Moribund condition* - all surviving ——— rats-killed by day 10

*Note that the final product rats were also in poor physical condition by day 10, but of less severity than the --- rats

↓ **BW** - 27% for drug; 31% for ---- - relative to baseline

↑ BUN, potassium, creatinine, bilirubin, GGT, AST, ALT, amylase - monomer & final product
↓ glucose, cholesterol - ——— & final product

Kidney - severe necrosis of tubular epithelial cells & tubular degeneration - ----- & final product
Liver - pigmentation/increased Kupffer cells; inflammatory cells; dilation of hepatic sinusoids - ——— & final product

Aggregates: no toxicities

Based on the physical condition of the rats, it appears that the ——— was slightly more toxic than the final product. Data suggest that the hepatic/renal toxicity is related to the ——— content of the preparation, and not to the ———
The sponsor cites a small PK study performed using the final product, ——— and ——— Sera levels, analyzed via ELISA (able to detect ——— $DAB_{389}IL-2$), showed that the ——— forms were rapidly cleared from the blood, while measurable sera levels of ——— remained:

Test Grow	5 min	15 min
Final Drug Product	2102 ± 257	1480 ± 81
———	2121 ± 273	1955 ± 576
———	199 ± 68	153 ± 64
———	318 ± 315	217 ± 21
———	97 ± 0	62 ± 13

4. Immunogenicity of $DAB_{389}IL-2$ Final Drug Product, cc-- and ——— Administered IV to Female Rats

Species: SD ♀ rats
Dose Levels: 0, 25 μg/kg/day
Route/Duration: IV/days 1-5; 22-26

Findings:
Abs - via ELISA
Day 19 - ——— - 50% positive (low/moderate)
——— & final drug product - 0-20% positive (low)

Day 40 - , ——— - 100% positive (moderate/high)
Final drug - 100% positive (low/moderate/high)
——— --- - 90-100% positive (low/moderate/high)

Neutralizing Abs - via an in vitro cell-based (C91/PL cells) assay (day 40 only)
Day 40 - Final drug &——— - 0% positive
——— - 30% positive (low/moderate)
-- - 55% positive (low/high)

Purified ——— was more immunogenic (55% neutralizing Abs) than the final product (0% neutralizing Abs)

Comments:
- [Per the sponsor] Polysorbate 20 present in the final drug is removed by the process used to produce the ——— material. Possibly removal of the ——— results in an effect on antigenic processing of the ——— However, the ——— also do not have polysorbate 20 present, yet did not display-enhanced immunogenicity.

- The amount of ——— in the final preparation has been increased on a relative mass basis. This ——— is likely responsible for the hepatic and renal toxicities.

Reproduction/Teratology Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. Placental Transfer of [^{35}S]$DAB_{389}IL-2$ in SD Rats; report #96025; performed at Seragen (non-GLP); ——— , 6/96; **vol 16**

Reproduction/Teratology Studies

1. Placental Transfer of [^{35}S]DAB$_{389}$IL-2 in SD Rats
Methods: Time-mated ♀ Hsd:SD rats (~GD 19) were singly IV injected at 25 μg/kg of [^{35}S]DAB$_{389}$IL-2, followed by kill at 1 and 24 hrs postdose. The embryos, placenta, amniotic fluid & sac, and the liver and kidneys of the dam were assayed for radiolabel. Sera from the dam were assayed for DAB,,, IL-2 levels by bioassay and anti-fragment B product ELISA.

Findings:
1 hr - liver = -21%; kidneys = -3% radiolabel detected
24 hrs - liver = -10%; kidneys = -1.2% radiolabel detected

Tissue/plasma (T/P) ratio - 1.0 (kidney & liver) at 1 hr - i.e., at steady state
T/P = 1.7 (liver), 1.3 (kidney) at 24 hrs - reflective of tissue accumulation

Embrvo - contained -1% of total dose at 1/24 hrs

T/P ratio of 0.3 at 1 hr [plasma levels higher than embryo - i.e., not getting through the placental

T/P = -1.0 - steady state - 24 hrs
Whole blood had -5.1% of total radiolabel, but no active material detected - probably reflective of ^{35}S breakdown products

Safetv Pharmacology Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. The Acute Cardiovascular Effects of ——— Administered IV to Conscious Male SD Rats; report #96149; performed at ——— (per GLP); lot ——— 11/96; vol 21

2. Measurement of Capillary Permeability Following IV Administration of DAB$_{389}$IL-2 to Naive and Preimmune SD Rats; report #96151; performed at Seragen (per GLP); lot ——— 4/97; vol 22

3. Estimation of Glomerular Filtration Rate and Renal Plasma Flow Following IV Administration of $DAB_{389}IL-2$ to SD Rats; report #96152; performed at Seragen (per GLP); lot [redacted] 5/97; vol 22

4. Plasma Cytokine Levels Following IV Administration of $DAB_{389}IL-2$ to SD Rats; report #96153; performed at Seragen (per GLP); lot [redacted]; 5/97; vol 22

Safety Pharmacology Studies

1. The Acute Cardiovascular Effects of [redacted] Administered IV to Conscious Male SD Rats
Species: SD ♂ rats (4/grp)
Dose Levels: 0, 14.8, 53.2, 149 kg/kg/day
Route/Duration: IV/5 days

Methods: Clinical signs, BWs, blood pressure, heart rates were measured

Findings:
Postdose #1
↑ systolic pressure (-8 mm Hg) - DAB grps
↑ MAP - DAB grps

Postdose #4
1/4 found dead prior to dosing - 149 kg/kg

Postdose #5
1/3 found dead prior to dosing - 149 μg/kg
↓ systolic, diastolic, MAP (-50%;) - 149 μg/kg

2. Measurement of Capillary Permeability Following IV Administration of $DAB_{389}IL-2$ to Naive and Preimmune SD Rats
Species: Hsd:SD ♀ rats (3/grp)
Dose Levels: 0, 25 μg/kg/day in naive & preimmune rats
Route/Duration: IV/see table
Preimmune rats - with $DAB_{389}IL-2$ [50 μg/rat/day], SC injection, for 4 days, at -3 weeks prior to IV dosing

Group Designations and Treatment

Group No.	Test Article	Immune status	No. of Animals	$DAB_{389}IL-2$ Dose Level (μg/kg/d)	Dose Vol. (mL/kg/d)	Days of Dosing	Test Day
1	Saline	naive	3	0	1	1	1
2	$DAB_{389}IL-2$	naive	3	25	1	1	1
3	$DAB_{389}IL-2$	naive	3	25	1	1-10	10
4	DAB & 2	preimmune	3	25	1	1	1
5	$DAB_{389}IL-2$	preimmune	3	25	1	1-10	10
6	IL-2	naive	3	NA*	NA*	1-2	3

* 400,000 U of IL-2 was given IP, three times pa dry. for two days, in a dose volume of 0.2 mL.

Methods: After the last dose of $DAB_{389}IL-2$ (at 15 mins) or IL-2 (at 12 hrs), ^{125}I-BSA was IV injected into each rat, followed (2 hrs later) by kill and collection of liver, kidneys, lungs, spleen, and ears.
Clinical signs, BWs, Ab titers, tissue water content, and permeability index [amount of radiolabel "leakage" into the interstitial spaces of target organs (kidneys, liver, lungs, spleen) and the peripheral vasculature (ears)] were evaluated.

Findings:
Hypoactivity, cyanosis - preimmune rats - postdose #1/2 - resolved within 30 mins - **noted in etudy #17 (Toxicology section) in the preimmune rats**

Ruffled fur - preimmune rats - day 10

↓ BWs - 10-day regimen - preimmune (days l-4) & naive rats (days l-10)

↑ Ab titers - preimmune rats - to $DAB_{389}IL-2$ only

↑ tissue water content - ears of preimmune rats (l-day regimen) - edema?
↓ tissue water content - ears, kidneys of naive rats (10-day regimen) - due to ↓ BWs; kidney toxicity

↑ permeability index - ears, lungs, spleen - 50-100% - IL-2 rats

↑ permeability index - ears (30%), lungs (30%), spleen (20%) - naive rats (1/10-day regimen)

↑ permeability index - lungs (20%), spleen (20%); ↓ kidney (40%) - preimmune rats (lo-day regimen)

3. Estimation of Glomerular Filtration Rate and Renal Plasma Flow Following IV Administration of $DAB_{389}IL-2$ to SD Rats
Species: Hsd:SD ♀ rats (5/grp)
Dose Levels: 0, 25 μg/kg/day
Route/Duration: IV/single dose or days l-10

Methods: Fifteen mins after the last dose, rats were IV injected with either ^{3}H-inulin or ^{14}C-PAH, followed by blood collection and counting of radiolabel.

Findings:
Ruffled fur - DAB rats
Numerous rats died/were euthanized during blood sampling.
↓ BWs - DAB rats (10-day regimen)

	Dose Davs	GFR (mL/min/q KW)	RPF (mL/min/q KW)
Control	1	0.80 ±0.100	3.12 ±0.699
25 μg/kg	1	1.28 f0.340	3.67 f0.808
25 μg/kg	10	0.37 ±Q.058	2.40 ±0.572

The decreased RPF is reflective of the renal toxicity seen with $DAB_{389}IL-2$. The sponsor suggests a possible change in renal hemodynamics, i.e., tubuloglomerular feedback.

Comment:
● [Per the sponsor] Inulin distributes into extracellular fluid, but does not cross membranes to enter intracellular fluid, thus the only route for its excretion is via the urine. Inulin is not reabsorbed by the renal tubules, so all excretion is via glomerular filtration. Plasma clearance = GFR [glomerular filtration rate].

● [Per the sponsor] p-aminohippuric acid [PAH] is cleared mainly by renal tubular excretion, thus all PAH that enters the kidney is cleared in a single pass, so renal venous PAH levels are zero. Plasma clearance of PAH provides an estimate of renal plasma flow (RPF).

4. Plasma Cytokine Levels Following IV Administration of $DAB_{389}IL-2$ to SD Rats
Species: Hsd:SD ♀ rats (5/grp)
Dose Levels: 0, 50 μg/kg/day
Route/Duration: IV/single dose or days l-10

Methods: In addition to the DAB grps, a grp of rats were IP. injected with 500,000 IU of IL-2 (Chiron), tid, on days l-3 and bid on day 4. Animals were bled predose and postdose.

Findings:
Ruffled fur - DAB rats (IO-day regimen)
↓ BWs - DAB rats (lo-day regimen)

No consistent effect on levels of TNFα, IL-1β, or IFNΓ noted.

Mutacfenicitv Studies

List of Studies:
Note that the dates presented with each **study are the dates the report was issued, not the date** of **study completion.**

1. The Effect of ——— on the Induction of Reverse Mutations in Salmonella typhimurium and E. coli Using the Ames Test; report #96110; performed at ——— (per GLP); lot ——— 7/96; vol 20

2. The Effect of --- on the In Vitro Induction of Chromosome Aberration in Chinese Hamster Ovary Cells; report #96109; performed at ——— (per GLP); lot ——— 8/96; vol 20

In Vitro Bacterial Svstem

1. The Effect of ——— on the Induction of Reverse Mutations in Salmonella typhimurium and E. coli Using the Ames Test
S. typhimurium strains TA1535, TA1537, TA98, and TA100 and E. coli strain WP2uvrA were incubated with and without S9, with 0.9375, 1.875, 3.75, 7.5, or 15 μg/plate of DAB_{389}IL-2. There was no induction of bacterial revertants in any of the assays.

In Vitro Mammalian Svstem

2. The Effect of -on the In Vitro Induction of Chromosome Aberration in Chinese Hamster Ovary Cells
CHO cells exposed to 0.01, 1.88, or 3.31 μg/mL of DAB_{389}IL-2 with or without S9 (metabolic activation) for -4 hrs, did not affect the number of cells with chromosomal aberrations compared to the positive controls [mitomycin C in nonactivated assays and cyclophosphamide in the activated assays]. There was no increase in the incidence of cells with diplochromosomes.

CONCLUSION:

The proposed clinical indication [per the package insert] for DAB_{389}IL-2 is for the treatment of patients with CTCL which is persistent or recurrent despite prior therapy. The proposed treatment regimen [per the package insert] is IV infusion (at least 15 mins) of — μg/kg/day ONTAK™ for 5 consecutive days. Courses may be repeated every 21 days [the maximum number of courses given clinically was 11].

This fusion protein utilizes the cytotoxic action of diphtheria toxin [DT], as well as the cell targeting ability for the IL-2R. Following the binding of the mature fusion protein of 58 kD to the IL-2R on cells, the protein is [redacted] This results in [redacted] and resulting in cell death. The first fusion protein developed by Seragen was $DAB_{486}IL-2$. This protein was used in Phase I/II clinical trials (124 patients, including 36 with CTCL). A more potent fusion protein - $DAB_{389}IL-2$ - was formulated, and following preclinical testing of both agents, the present product was used in clinical trials.

$DAB_{389}IL-2$ is a potent cytotoxic agent for cell lines and activated lymphocytes which express the high affinity IL-2R (lowest $IC_{,,} = 2 \times 10^{-12}$ M). Cells that do not express IL-2R or express the p55 or only the p75 and p64 subunits of the IL-2R, are not sensitive to the fusion protein. In addition, $DAB_{389}IL-2$ was equally cytotoxic for T and B cells of human, monkey, rat, and mouse origin that expressed IL-2R. $DAB_{389}IL-2$ was 4 to 30-fold more potent than $DAB_{,,}$ IL-2, depending on the cell type used. The sponsor attributes this higher potency to the greater affinity of $DAB_{,,}$ IL-2 for the high affinity IL-2R & the shorter contact time needed for irreversible binding to the receptor. The kinetics of the inhibition of protein synthesis were similar for both materials.

The human IL-2R is present in three forms: low, intermediate, and high affinity. The low affinity IL-2R is made up of a 55 kD (p55, Tac, α chain, CD25) protein (K_d = -10 nM) and is unable to mediate internalization of bound ligand or signal transduction. The 64 kD (p64, Γ chain) protein and the 75 kD (p75, β chain, CD122) protein separately, are limited in ability to bind IL-2R, but when combined, β-Γ heterodimer can bind IL-2R with intermediate affinity (K_d = -1 nM). Both β and Γ chains are needed for the receptor to internalize ligand and mediate signal transduction. The low affinity IL-2R is made up of p55 and the intermediate IL-2R of p75/p64. Noncovalent complexing of all three chains results in a high affinity complex (K_d = -10 pM). Expression of this complex in humans is associated with immune activation.

The high affinity complex is the biologically relevant form of the IL-2R on mature activated T cells, with expression generally restricted to activated T cells, B cells, and monocytes. When $DAB_{389}IL-2$ binds to all three forms of the IL-2R, cells that express only the low or intermediate affinity receptor are at

least 100-fold less sensitive to the fusion protein. Once DAB_{389}IL-2 binds to the surface of the high affinity form of the IL-2R, the molecule is [redacted] and [redacted], causing cell death.

The activity of DAB,,, IL-2 was assessed in vitro via the ability of the molecule to inhibit protein synthesis of various cell types, both tumor cells and normal cells, that did/did not express the IL-2R. The IC,, concentration, determined via the incorporation of radiolabeled leucine, ranged from 0.1-12 ng/mL for activated human CD4+ and CD8+ cells, activated human PBMCs, and tumor cells bearing the IL-2R. The addition of IL-2 competitively blocked DAB_{389}IL-2 activity, while IL-4 did not.

In vivo studies were conducted with the CP3 murine model of IL-2R expressing malignancy [which expresses the p55/p75/p64 receptors]. Animal survival was prolonged and the onset of clinical signs of malignancy were prevented/delayed at DAB_{389}IL-2 doses up to 40 μg/kg/day for 10 days. The use of DAB,,, molecules with dysfunctional domains [redacted] was not effective in the CP3, EL-4, and P388 murine tumor models - thus supporting the specificity of DAB,,, IL-2 for IL-2R expressing tumors. However, administration of [redacted] (which binds poorly to IL-2R due to a deletion in the IL-2 part of the molecule) to naive normal rats resulted in toxicities that were similar to those seen with DAB_{389}IL-2, but of a lower frequency and severity. Thus some of the toxicities observed probably reflect non-specific activity related to the toxin portion of the molecule.

The evaluation of various dosing regimens in the CP3 model showed that episodic regimens (3 days/wk x 3 wks or 5 days/wk x 2 wks) were as effective as daily dosing for 10 days. Preimmune (to the DT) tumor-bearing mice also displayed prolonged survival when given DAB_{389}IL-2, although not as effectively as naive tumor-bearing mice. Naive mice bearing the CP3 tumors displayed, toxicities identical to those seen in normal naive mice [i.e., weight loss, abnormal renal and liver chemistries, and hepato-cellular and renal tubular epithelial cell necrosis], but with a lower incidence and severity.

As DAB_{389}IL-2 is not species specific, mice, rats, guinea pigs, rabbits, and cynomolgus monkeys were used in the performance of the toxicology studies. A single IP injection showed a NOAEL of <7.5 μg/kg in mice and <150 μg/kg in guinea pigs, with weight loss noted in all DAB_{389}IL-2 groups.

Although the MTD varied for each species (rabbit & monkey < mouse & rat), toxicity was consistently seen in two **major** organs - the liver and kidney. In repeated dose studies performed in preimmune and naive mice [daily for 10 days], naive mice displayed more severe toxic effects compared to preimmune mice. Such findings included weight loss, **elevated liver chemistries,** and hepatocellular and renal tubular necrosis. Repeated doses [10-day regimen] administered to naive mice and/or rats ranged from 25-108 μg/kg/day. A NOEL was not **achieved.**

In addition to displaying the aforementioned observations, death occurred in rabbits IV injected at 31 μg/kg/day for 7 days due to the severity of the toxicities. The dehydrated and **weak** animals exhibited many chemistry and hematology abnormalities reflective of their poor physical condition, as well as a result of target organ toxicity. The kidneys [tubule degeneration, eosinophilia, and dilatation, focal mineralization]; liver [hepatocellular necrosis]; lungs [interstitial inflammation]; and adrenals [necrosis, inflammation, leukocyte infiltrates, hemorrhage] were affected. A NOEL was not **achieved.**

Daily IV injection in naive cynomolgus monkeys at levels up to 25 μg/kg/dose for 30 consecutive days or in 2-week segments, interrupted by a 2-week nondosing interval, resulted in severe toxicities in all DAB_{389}IL-2 groups, causing deaths in 5/17 animals at 25 μg/kg. Medication was needed to counteract the weight loss/dehydration/diarrhea seen in all DAB_{389}IL-2 groups (doses from 2.5-25 μg/kg/dose). Additional dose-related adverse findings were similar to those noted in rabbits and rodents, but with higher severity. Two 25 μg/kg monkeys also exhibited meningeal hemorrhage and two monkeys (2.5 and 25 μg/kg) displayed hepatic vasculitis. A NOEL was not achieved. A total of 11/206 (5%) patients experienced thrombotic events, with 6/11 showing superficial thrombophlebitis.

Hepatic multifocal mononuclear cell infiltrates were seen in naive rats dosed at 25 μg/kg/day for 14 days, but were absent at doses of 100 μg/kg/day for 3 days. Findings observed in both dose groups for naive and preimmune rats included infiltrates in the kidney, basophilic/dilated renal tubules, splenic lymphoid hyperplasia, and inflammation in the lungs - with greater severity and longer persistence in naive animals.

An important aspect of the assessment of the preclinical toxicities seen with DAB,,,IL-2 was the relationship between the development of anti-DAB,,,IL-2 antibodies and the incidence, severity, and duration of the adverse effects noted in animals evaluated. Administration of DAB,,,IL-2 to mice 5 times/week for 4 weeks resulted in greater tolerance to the molecule compared to rats injected with the same doses according to the identical

dosing regimen. Mice also developed antibodies much quicker [by day 153 than did the rats [by day 301. This finding was corroborated several times via studies using preimmune and naive rodents. In each case, the preimmune mice/rats tolerated the $DAB_{389}IL-2$ toxicities better than the concurrently dosed naive animals. Even with the variability in the data, it appeared that the presence of higher titers of **anti-$DAB_{389}IL-2$** antibodies increased the clearance rate of the molecule. The liver and kidneys were the primary sites of in vivo distribution of $DAB_{389}IL-2$ outside of the vasculature. The intact molecule is converted to an active metabolite of **$DAB_{389}IL-2$** via proteolytic cleavage in vivo and injection of this nicked molecule to rats resulted in greater renal and hepatic toxicity and faster disappearance from the serum compartment. The presence of antibodies slowed the conversion process from intact to nicked molecule.

Of note, two studies showed that preimmune rats exhibited hypoactivity, ataxia, and/or cyanosis following the initial doses of $DAB_{389}IL-2$ at ≥25 μg/kg/dose. The onset and duration of the transient signs were dose-related. The sponsor offers no explanation for these findings. A total of 5/206 (-2%) patients have experienced acute hypersensitivity reactions which were moderate in severity, immediately following IV administration of $DAB_{389}IL-2$. Signs included hypotension, back pain, rash, dyspnea, chest tightness, and tachycardia. The reactions were relieved with slowing/stopping the infusion and antihistamines/ corticosteroids. The PI contains a warning regarding this adverse effect.

Naive rats IV injected at 75 μg/kg/day for 10 days with final $DAB_{389}IL-2$ product, [redacted] [from the product], or [redacted], displayed no toxicities due to the [redacted] Based-on-the physical condition of the rats, it appears that the [redacted] was slightly more toxic than the final product. The data strongly that the hepatic/renal toxicity is related to the -- content of the preparation, and not to the [redacted] Sera levels, showed that the [redacted] forms were rapidly cleared from the blood, while measurable sera levels of .-remained. This raises the question of the relative contribution of [redacted] in the formulated product. Preclinical studies performed appeared to show a general trend toward a lower NOAEL in parallel with the generation of newer lots of $DAB_{389}IL-2$. Of note is that these studies were not included in the BLA submission, but were from IND [redacted]

According to the FDA product reviewers, there are several concerns with the clinical grade product: 1) the profile of product-related degradents is not well characterized; 2) the limits for the amount of [redacted] is not specified; 3) characterization of [redacted] is

incomplete; and 4) there is uncertainty as to the actual amount of biologically active drug in the final product. The sponsor has received numerous question/comments regarding these and other product issues. Note that additional in vitro or in vivo nonclinical studies - unspecified at this point - may be required, pending the sponsor's response to the issued letter.

Administration of $DAB_{389}IL\text{-}2$ resulted in increases in the tissue water content of the ears of preimmune rats and in the permeability index [i.e., protein "leakage" into interstitial spaces] of the spleen, lungs, and ears of naive and preimmune rats (20-30%). The tissue water content of the ears and kidneys decreased in naive rats - likely due to weight loss and renal toxicity. Note that naive rats displayed the findings at earlier dose intervals. Rats given IL-2 showed much higher increases in permeability index of ears, spleen, and lungs (50-100%).

Decreased renal plasma flow and glomerular filtration rates were noted in naive rats IV injected with 25 $\mu g/kg/day$ $DAB_{389}IL\text{-}2$ for 10 days. The IC,, for protein synthesis inhibition of $DAB_{389}IL\text{-}2$ for rat hepatocytes, Kupffer cells, renal proximal tubule epithelial cells was $\sim 10^{-8}M$ (576 ng/mL). Note that single bolus doses of $DAB_{389}IL\text{-}2$ in naive rats approached this sera level. Protein synthesis was not inhibited in primary human cell types [i.e., pulmonary artery and dermal microvascular endothelial cells; proximal tubular and bronchial epithelial cells; etc...] at 576 ng/mL.

Single IV administration of 25 $\mu g/kg$ of radiolabeled $DAB_{389}IL\text{-}2$ to pregnant rats on gestation day 19 showed predominant accumulation of radiolabel in the liver and kidneys of the dam at 1 and 24 hrs postdose. The embryo only contained -1% of the total dose at these intervals and the embryonic tissue/maternal plasma ratios were 0.3 at 1 hr, indicative of minimal/no placental passage. No developmental toxicity studies were performed with this product.

No mutagenic potential was exhibited via in vitro bacterial and mammalian systems.

The preclinical toxicity data submitted by the sponsor appear to correlate with the adverse clinical events that are of concern in the trials. The predominant toxic effects displayed by the animals involved the liver, kidney, spleen, and lungs. Many of the adverse findings exhibited by humans - hepatic and renal toxicities, vascular leak syndrome, thrombotic events, and hypersensitivity reactions - involved these target organs. Although of low incidence, the occurrence of vasculitis (liver, monkey) and thrombi (brain, rat) was noted. Many of the in vivo findings noted seem to be reflective of IL-2-like toxicities.

The package insert contains specific information regarding the clinical toxicities; it will be modified to better reflect the preclinical data.

The preclinical data support use of the product, ONTAK™, for the indicated patient population and dosage regimen. However, additional in vitro or in vivo studies may be needed pending the sponsor's response to the letter comments issued by the product reviewers. An amendment to this review will be issued, as necessary, to address those issues that may arise.

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Key Words: $DAB_{486}IL\text{-}2$; $DAB_{389}IL\text{-}2$; toxicity; preclinical studies; cutaneous T-cell lymphoma; psoriasis; diphtheria toxin fusion protein; IL-2 toxicity; hepatic toxicity; renal toxicity; Abs; lymphopenia

concurrences:
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